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BROILER PERFORMANCE AND MINERAL UTILIZATION OF ENZYME-SUPPLEMENTED DEFATTED RICE BRAN DIET DURING HEAT STRESS

A Dissertation
Presented for the
Doctor of Philosophy
Degree
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Ornprapun Puminn
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This dissertation is dedicated to the King of Thailand

His Majesty King Bhumibol Adulyadej

His deep interest in Thai people's welfare and his devoted hard work in order to improve life of Thai people is always my inspiration

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ABSTRACT

Defatted rice bran is a by-product from the solvent-extraction of rice bran. It contains high amounts of phytate and non-starch polysaccharides, which are considered to be two major antinutritional factors that limit the use of rice bran in poultry diets. Two experiments were conducted to evaluate broiler performance and mineral utilization of defatted rice bran diets supplemented with commercial enzyme phytase alone or a combination of phytase and xylanase under two different environmental temperatures. In the first experiment, six replicate groups of day-old chicks were assigned to five dietary treatments comprised of a control corn-soybean meal diet with 25% corn starch and four diets containing two levels of defatted rice bran (10 and 25%) with or without phytase (500 units/kg diet) supplementation. In experiment 2, five replicate groups of chicks were assigned to dietary treatments similar to first experiment, but using both phytase (500 units/kg diet) and xylanase (1000 units/kg diet) instead of phytase alone plus an additional basal corn-soybean meal based diet. Available phosphorus in diets containing phytase was calculated to be 0.1% reduction from the requirement for broilers. On day 21, some birds were slaughtered and tibias harvested for mineral analysis. The remaining birds were transferred to either a thermoneutral temperature chamber (23.9^{o}C) or a heat stress chamber (23.9-35^{o}C, diurnal). Digesta retention time was determined on day 35. Birds were slaughtered at seven weeks of age, tibias harvested, and intestines examined.

In both experiments, using up to 25% defatted rice bran in diets was not detrimental to broilers. The beneficial effect of high fat level can overcome some problems associated with high phytate phosphorus and non-starch polysaccharide in

defatted rice bran. As a result, birds fed 25% defatted rice bran diet had the highest body weight gain. Supplementing with enzymes improved feed conversion ratio of 3-week-old chicks fed 10% defatted rice bran diet. No significant differences were found in weight percentage of most parts of the carcass, as well as in morphology of the duodenum and the jejunum examined by villi height and crypt depth measurements. Abdominal fat was increased in birds fed 25% defatted rice bran diets. Tibia phosphorus, zinc and manganese content of chicks fed 10% defatted rice bran diet were improved by supplemental phytase alone, while tibia calcium, phosphorus, and manganese content of chicks were improved when both enzymes were added to 25% defatted rice bran diet. However, only the improvement of tibia phosphorus of 7-week-old birds was detected when phytase was added to 25% defatted rice bran diet or when both enzymes were added to 10% defatted rice bran diet. Although percent phosphorus in the excreta was decreased in both experiments in birds fed 25% defatted rice bran diets supplemented with enzyme, phosphorus retention of birds did not improve by using phytase alone. Since the price of rice bran was cheaper than other major ingredients, use of 25% defatted rice bran diet lowered feed cost per weight gain of broilers. From the growth performance and feed cost per weight gain of birds, supplemental enzyme did not alleviate the adverse effects of heat stress.

(Key words: defatted rice bran, phytase, xylanase, heat stress, broilers)

TABLE OF CONTENTS

Part		Page
I.	INTRODUCTION	1
	Research Objectives	8
	References	9
II.	LITERATURE REVIEW	14
	Rice Bran	15
	Phytate	21
	Non-Starch Polysaccharides	25
	Cellulose	25
	Hemicellulose	26
	β-glucans	30
	Pentosans	31
	Use of Enzymes in Poultry Diets	33
	Phytase	34
	β-glucanase and Xylanase	37
	Secondary Benefit of Using Enzymes in Poultry Diets	39
	Effect of Heat Stress in Poultry	41
	References	46
III.	EXPERIMENT 1: BROILER PERFORMANCE AND MINERAL UTILIZATION OF PHYTASE-SUPPLEMENTED DEFATTED RICE BRAN DIET DURING HEAT STRESS	58
	Abstract	59
	Introduction	60
	Materials and Methods	62
	Chickens and Diets	62
	Temperature Treatments	67
	Digesta Retention Time Measurement and Excreta Collection	69
	Processing of Birds	69
	Villi Height and Crypt Depth Measurements	70
	Tibia Mineral Determination	71
	Calcium and Phosphorus Retention Determination	71
	Apparent Metabolizable Energy Determination	73
	Feed Cost per Weight Gain Determination	73
	Experimental Design and Statistical Analysis	74
	Results	74
	Growth Performance	74
	Water Intake and Digesta Retention Time	76
	Carcass Quality	82
	Gross Anatomical Observations of Intestine	86

Intestinal Villi Height and Crypt Depth……..……. 86
Tibia Mineral Content……... 86
Calcium and Phosphorus Retention……...……. 93
Apparent Metabolizable Energy……...……. 98
Feed Cost per Weight Gain……...……. 102
Discussion……..……. 102
Conclusion……. 118
References……. 119

IV. EXPERIMENT 2: BROILER PERFORMANCE AND MINERAL UTILIZATION OF PHYTASE AND XYLANASE-SUPPLEMENTED DEFATTED RICE BRAN DIET DURING HEAT STRESS……..……. 125
Abstract…….. 126
Introduction……...…… 127
Materials and Methods……...……... 130
Chickens and Diets…… 130
Temperature Treatments…… 134
Digesta Retention Time Measurement and Excreta Collection…… 136
Processing of Birds…… 136
Villi Height and Crypt Depth Measurements…… 137
Tibia Mineral Determination……...…….. 138
Calcium and Phosphorus Retention Determination……..…… 139
Apparent Metabolizable Energy Determination…...……. 139
Feed Cost per Weight Gain Determination……...…… 139
Experimental Design and Statistical Analysis……...…… 140
Results……...…… 140
Growth Performance……. 140
Water Intake and Digesta Retention Time……...……. 146
Carcass Quality……...…… 149
Gross Anatomical Observations of Intestine……. 149
Intestinal Villi Height and Crypt Depth……...……. 153
Tibia Mineral Content……... 153
Calcium and Phosphorus Retention……...…… 161
Apparent Metabolizable Energy……...……. 166
Feed Cost per Weight Gain……...……. 169
Discussion……..…… 172
Conclusion……. 183
References……. 185

V. SUMMARY AND CONCLUSIONS…… 190

VITA …… 195

LIST OF TABLES

Table Page

PART II
LITERATURE REVIEW

1. Proximate analysis of rice bran and polish (dry basis)……. 16
2. Amino acids content of rice bran ……..……..……..…… 16
3. Minerals and vitamins content of rice bran……...……...…… 18
4. Fatty acid composition of rice bran…….. 18

PART III
EXPERIMENT 1: BROILER PERFORMANCE AND MINERAL UTILIZATION OF PHYTASE-SUPPLEMENTED DEFATTED RICE BRAN DIET DURING HEAT STRESS

1. Composition of defatted rice bran……. 63
2. Composition of starter diets (%)……...……. 64
3. Composition of grower diets (%)…….……. 65
4. Composition of finisher diets (%)..……..……..…… 66
5. Analyzed nutrient composition of experimental diets (%)…… 68
6. Body weight, weight gain, feed intake, and feed conversion ratio of 3-week-old broilers fed different experimental diets……..……...….. 75
7. Body weight at 7 week of age, weight gain, feed intake, and feed conversion ratio during 3-7week of age of broilers fed different experimental diets under thermoneutral and heat stress conditions…….…… 77
8. Total weight gain, feed intake, and feed conversion ratio during 0-7 week of age of broilers fed different experimental diets under thermoneutral and heat stress conditions…….. 78
9. Main effect means of daily water intake and water:feed consumption ratio of broilers during 3-7 week of age, and digesta retention time of 5-week-old broilers fed different experimental diets under thermoneutral and heat stress conditions……..…… 80
10. Carcass, abdominal fat, and intestinal weight as percentages of live weight of 7-week-old broilers fed different experimental diets under thermoneutral and heat stress conditions……..…… 84
11. Breast, legs, thighs, and wings weight as percentages of carcass weight of 7-week-old broilers fed different experimental diets under thermoneutral and heat stress conditions…….. 85
12. Duodenum, jejunum, ileum, ceca, and colon weight as percentages of intestinal weight of 7-week-old broilers fed different experimental diets under thermoneutral and heat stress conditions……...….. 87

13. Duodenal villi height, crypt depth, and villi height:crypt depth ratio of 7-week-old broilers fed different experimental diets under thermoneutral and heat stress conditions…….…… 88
14. Jejunal villi height, crypt depth, and villi height:crypt depth ratio of 7-week-old broilers fed different experimental diets under thermoneutral and heat stress conditions……..… 89
15. Dry matter, ash, calcium and phosphorus content, and calcium:phosphorus ratio in tibia of 3-week-old broilers fed different experimental diets……..…. 90
16. Zinc and manganese content in tibia of 3-week-old broilers fed different experimental diets……..…… 90
17. Dry matter, ash, calcium and phosphorus content, and calcium:phosphorus ratio in tibia of 7-week-old broilers fed different experimental diets under thermoneutral and heat stress conditions……...…… 91
18. Zinc and manganese content in tibia of 7-week-old broilers fed different experimental diets under thermoneutral and heat stress conditions…….…. 94
19. Percent calcium and phosphorus in excreta, and their retention by 5-week-old broilers fed different experimental diets under thermoneutral and heat stress conditions…….. 96
20. Apparent metabolizable energy of different experimental diets of 5-week-old broilers under thermoneutral and heat stress conditions……..…. 100
21. Feed cost (dollar) per pound diet of different experimental diets……..... 103
22. Feed cost per weight gain of broilers fed different experimental diets during 0-3 week of age……..…… 103
23. Feed cost per weight gain of broilers fed different experimental diets during 3-7 and 0-7 week of age under thermoneutral and heat stress conditions……. 104

PART IV
EXPERIMENT 2: BROILER PERFORMANCE AND MINERAL UTILIZATION OF PHYTASE AND XYLANASE-SUPPLEMENTED DEFATTED RICE BRAN DIET DURING HEAT STRESS

1. Composition of starter diets (%)……...…… 131
2. Composition of grower diets (%)…….…… 132
3. Composition of finisher diets (%)….……...…….…… 133
4. Analyzed nutrient composition of experimental diets (%)…… 135
5. Body weight, weight gain, feed intake, and feed conversion ratio of 3-week-old broilers fed different experimental diets……...……..….. 141
6. Body weight at 7 week of age, weight gain, feed intake, and feed conversion ratio during 3-7 week of age of broilers fed different experimental diets under thermoneutral and heat stress conditions……...……..…… 143
7. Total weight gain, feed intake, and feed conversion ratio during 0-7 week of age of broilers fed different experimental diets under thermoneutral and heat stress conditions…….. 144

8. Main effect means of daily water intake and water:feed consumption ratio of broilers during 3-7 week of age, and digesta retention time of 5-week-old broilers fed different experimental diets under thermoneutral and heat stress conditions…… 147
9. Carcass, abdominal fat, and intestinal weight as percentages of live weight of 7-week-old broilers fed different experimental diets under thermoneutral and heat stress conditions…… 151
10. Breast, legs, thighs, and wings weight as percentages of carcass weight of 7-week-old broilers fed different experimental diets under thermoneutral and heat stress conditions…….. 152
11. Duodenum, jejunum, ileum, ceca, and colon weight as percentages of intestinal weight of 7-week-old broilers fed different experimental diets under thermoneutral and heat stress conditions…….. 154
12. Duodenal villi height, crypt depth, and villi height:crypt depth ratio of 7-week-old broilers fed different experimental diets under thermoneutral and heat stress conditions…… 155
13. Jejunal villi height, crypt depth, and villi height:crypt depth ratio of 7-week-old broilers fed different experimental diets under thermoneutral and heat stress conditions…… 156
14. Dry matter, ash, calcium and phosphorus content, and calcium:phosphorus ratio in tibia of 3-week-old broilers fed different experimental diets…….. 157
15. Zinc and manganese content in tibia of 3-week-old broilers fed different experimental diets…… 157
16. Dry matter, ash, calcium and phosphorus content, and calcium:phosphorus ratio in tibia of 7-week-old broilers fed different experimental diets under thermoneutral and heat stress conditions…… 158
17. Zinc and manganese content in tibia of 7-week-old broilers fed different experimental diets under thermoneutral and heat stress conditions…… 160
18. Percent calcium and phosphorus in excreta, and their retention by 5-week-old broilers fed different experimental diets under thermoneutral and heat stress conditions…….. 163
19. Apparent metabolizable energy of different experimental diets of 5-week-old broilers under thermoneutral and heat stress conditions…….. 167
20. Feed cost (dollar) per pound diet of different experimental diets……..... 170
21. Feed cost per weight gain of broilers fed different experimental diets during 0-3 week of age…… 170
22. Feed cost per weight gain of broilers fed different experimental diets during 3-7 and 0-7 week of age under thermoneutral and heat stress conditions……. 171

LIST OF FIGURES

Figure Page

PART II
LITERATURE REVIEW

1. Structure of phytic acid…..…….……...……...….. 22
2. The possible bonds of phytate……...…… 24
3. Structure of common non-starch polysaccharides……. 28

PART III
EXPERIMENT 1: BROILER PERFORMANCE AND MINERAL UTILIZATION OF PHYTASE-SUPPLEMENTED DEFATTED RICE BRAN DIET DURING HEAT STRESS

1. Villi height and crypt depth measurement using MetaMorph Imaging System……...… 72
2. Weight gain (WG) and feed intake (FI) of 7-week-old broilers fed different experimental diets under thermoneutral (TN) and heat stress (HS) conditions…….. 79
3. Water:feed consumption ratio of broilers during 3-7 week of age fed different experimental diets under thermoneutral (TN) and heat stress (HS) conditions…….. 81
4. Digesta retention time of 5-week-old broilers fed different experimental diets under thermoneutral (TN) and heat stress (HS) conditions…..……... 83
5. Amount of excreta (dry matter basis) as percentage of feed consumed by 5-week-old broilers fed different experimental diets under thermoneutral (TN) and heat stress (HS) conditions……..... 95
6. Percent calcium (Ca) and phosphorus (P) in excreta by 5-week-old broilers fed different experimental diets under thermoneutral (TN) and heat stress (HS) conditions……..… 97
7. Percent calcium (Ca) and phosphorus (P) retention by 5-week-old broilers fed different experimental diets under thermoneutral (TN) and heat stress (HS) conditions…….. 99
8. Apparent metabolizable energy of different experimental diets of 5-week-old broilers under thermoneutral (TN) and heat stress (HS) conditions……..…… 101

PART IV
EXPERIMENT 2: BROILER PERFORMANCE AND MINERAL UTILIZATION OF PHYTASE AND XYLANASE-SUPPLEMENTED DEFATTED RICE BRAN DIET DURING HEAT STRESS

1. Weight gain (WG) and feed intake (FI) of 7-week-old broilers fed different experimental diets under thermoneutral (TN) and heat stress (HS) conditions……...…. 145
2. Water:feed consumption ratio of broilers during 3-7 week of age fed different experimental diets under thermoneutral (TN) and heat stress (HS) conditions……..... 148
3. Digesta retention time of 5-week-old broilers fed different experimental diets under thermoneutral (TN) and heat stress (HS) conditions.……..…... 150
4. Amount of excreta (dry matter basis) as percentage of feed consumed by 5-week-old broilers fed different experimental diets under thermoneutral (TN) and heat stress (HS) conditions…… 162
5. Percent calcium (Ca) and phosphorus (P) in excreta by 5-week-old broilers fed different experimental diets under thermoneutral (TN) and heat stress (HS) conditions……..… 164
6. Percent calcium (Ca) and phosphorus (P) retention by 5-week-old broilers fed different experimental diets under thermoneutral (TN) and heat stress (HS) conditions…….……...…….. 165
7. Apparent metabolizable energy of different experimental diets of 5-week-old broilers under thermoneutral (TN) and heat stress (HS) conditions……..… 168

PART I

INTRODUCTION

The demand for poultry products has increased throughout all areas of the world following the growth of the human population. The increasing population of both poultry and people has brought a competition for use of cereal grains as poultry feed and human food ingredients. Corn, which is one of major ingredients in poultry feeds, has been increasing in price because of high demand. To decrease the cost of feed production, other sources of nutrients and by-products from agricultural processing manufacturers need to be considered.

Rice (*Oryza sativa L.*) is the major food grain for people consisting of about two-thirds of the world population, with 90% of the world's production grown in the Asian region (Saunders, 1986). Rice bran, a major by-product from the rice milling process, has been used as an ingredient in livestock feed. It is probably the most widely used cereal by-product available. The feeding value of rice bran used as an ingredient in poultry feed has been reviewed by Sayre et al. (1987) and Farrell (1994). Because of its high oil content (20%), rice bran is poorly metabolized by young birds (Warren and Farrell, 1990a). Rice bran oil is popularly used for cooking in many countries. It contains a high concentration of unsaturated fatty acids (80-85%) which easily become rancid, especially under warm and humid climatic conditions. Hussein and Kratzer (1982) found that the free fatty acid content of rice bran fat was increased from 13.7% before storage to 42.8% during 3-month storage period. Rice bran stored without addition of EDTA gave significantly lower broiler growth rate than either fresh or EDTA supplemented rice bran. The extraction of oil from rice bran has resulted in the availability of increasing amounts of defatted rice bran, which has been used for animal

feedstuff. The nutritional value of defatted rice bran is equal to the value of full fat rice bran in chicken diets when diets are adjusted for metabolizable energy by adding oil (Farrell, 1994).

Defatted rice bran contains a relatively high percentage of non-starch polysaccharides, with arabinose and xylose being predominant (Annison et al., 1995). This may have an adverse effect on the digestion of some dietary components. The main influence of non-starch polysaccharides in poultry diets is to increase viscosity of digesta and excretion of sticky droppings (Iji, 1999). High viscosity in intestinal content causes reduction in enzyme activities and nutrient absorption (Smits and Annison, 1996). It also enhances bacterial overgrowth (Salih et al., 1991) and bacterial fermentation due to undigested material accumulation in small intestine (Smits et al., 1997). Increasing viscosity due to dissolving of arabinoxylan in the intestinal tract results in a decrease the rate of nutrient diffusion (Fengler and Marquardt, 1988). Choct and Annison (1992) showed that pentosan extracted from wheat caused increased digesta viscosity, and reduced feed intake, weight gain, and feed conversion efficiency of broiler chickens. Increasing the viscosity at a given dietary concentration of carbohydrate gelling agent, hydroxyethyl cellulose, or increasing the dietary concentration at a given viscosity in rat led to increases in small intestinal length, mucosal DNA content, caecal weight, and colonic weight (Elsenhans and Caspary, 2000). Wet litter occured due to the water binding capacity of arabinoxylans in wheat (Bedford and Morgan, 1996).

About 90% of the phosphorus in rice bran is in the form of phytic acid or phytate (McCall et al., 1953). In rice, most of phytate is deposited in the germ and pericarp

(Reddy et al., 1989), which are the particles that have been detected in commercial rice bran (Barber and Benedito de Barber, 1985). Australian rice bran was found to contain phytate at levels of 23-40 g/kg (Warren and Farrell, 1991). Phytate phosphorus is unavailable to simple-stomach animals because of lack of the enzyme, phytase, in the gastrointestinal tract (Scott et al., 1982). The high phytate content can reduce availability of other minerals, such as calcium, magnesium, iron, and zinc, which can be complexed with phytate at physiological pH (Reddy et al., 1989; Ravindran, 1995). Phytate is a polyanionic molecule with six phosphate groups, therefore it can readily form insoluble complexes with divalent cations in weak acidic to neutral pH conditions and, consequently, reduce their availability in animals (Davies and Nightingale, 1975). Increasing dietary phytate had negative influence on body weight gain, feed intake, and feed conversion of broilers (Cabahug et al., 1999). A decrease in percentage of broiler tibia ash ($P<0.08$) occurred when the amount of defatted rice bran was increased in broiler diets (Adrizal et al., 1996). However, defatted rice bran could be used at up to 22.5% in broilers from 4 to 35 days of age when diets were supplemented with available phosphorus and fat. The reduction in tibia ash might relate to an adverse effect of phytate on calcium utilization (Nelson and Kirby, 1988). Warren and Farrell (1990b) found that osteoporosis occurred in laying hens on diets with 25% defatted rice bran with adequate concentrations of minerals for 6 weeks. Piliang et al. (1982) fed pullets with a diet contained 81.5% rice bran. Egg production was significantly lower than in pullets fed a standard breeder diet. The progeny of those pullets showed feather defects, which could be eliminated by adding zinc carbonate to the diet. Thompson and Weber (1981) also found that chicks fed diets-containing rice bran had lower zinc content of the tibia.

Trace minerals play very important roles in the growth and maintenance of tissues. However, research conducted concerning some trace minerals has been minimal. Minerals that are generally adequate or only slightly deficient in practical diets have traditionally been overlooked. Requirements for some of these minerals may vary because of the biological availability in practical diets. In the case of manganese, it has been shown that some ingredients reduce its availability in the inorganic form. At the same time, the biological availability of the chelated form of this mineral was increased when broilers were subjected to heat stress (Smith et al, 1995).

Rice bran is an excellent plant source of manganese (Scott et al., 1982) but the availability of manganese is low. The utilization of manganese might be impaired by some factors present in natural feed ingredients, such as phytic acid, fiber, and other mineral elements (Halpin and Baker, 1987). Manganese deficiency resulted in qualitative changes in proteoglycans present in epiphyseal growth plate cartilage of broiler chicks (Liu et al., 1994). Rice bran was shown to reduce tissue manganese deposition when dietary manganese was in excess of requirement (Halpin and Baker, 1986). Addition of rice bran to the diet also caused the reduction of manganese deposition in chick tibias, pancreas, and gallbladder (Thompson and Weber, 1981; Halpin and Baker, 1987). However, rice bran significantly increased tissue manganese concentrations when added to diets containing a level of manganese below the chick's requirement (Halpin and Baker, 1986).

The addition of exogenous fiber degrading enzymes, such as glucanases and xylanases, to poultry diets containing non-starch polysaccharides was shown to improve

the nutritional value of barley-, wheat-, and rye-based diets (Brenes et al., 1993a; Bedford and Morgan, 1996). The intestinal viscosity could be reduced with the addition of appropriate dietary enzymes (Choct et al., 1999). The reduction of viscosity improves the rate of nutrient diffusion, which is correlated with improved in weight gain and feed conversion efficiency of broiler chickens. The relative length and weight of the digestive tract and the pancreas also were reduced by enzyme supplementation in barley-based diet (Brenes et al., 1993a). Enzyme (β-glucanase/pentosanase complex) supplementation in young pullets fed hulless barley, naked oat, or rye diets had a beneficial effect on weight gain and feed intake but not on egg production (Brenes et al., 1993b). However, Farrell and Martin (1998a) found that addition of an enzyme mixture containing xylanase, α-amylase, β-glucanase, and protease to broilers fed rice bran diets had no benefit. Supplementation of phytase produced from a fungus *Aspergillus niger* to a corn-soybean diet with normal phosphorus level was found to increase tibial and toe ash contents of broilers. In a low phosphorus diet, additional phytase resulted in improved growth rate, relative retention of calcium and phosphorus, and bone mineralization (Ahmad et al., 2000). Phytase was also shown to improve the nutritive value of rice bran (Farrell, 1994), while tibia ash and growth rate was increased when phytase had been added to duck diet containing 20% rice bran (Farrell and Martin, 1998b).

Poultry production in many regions of the world is adversely affected by heat distress. Its effects are economically significant and geographically widespread. Tropical countries with near constant high temperatures are particularly vulnerable; thus there is a need for solutions that hinge on easily implemented techniques. Problems

associated with heat distress in broilers are well documented. The primary consequence of elevated growing temperature is reduction in feed intake (Dale and Fuller, 1980) in order to reduce metabolic heat production. Reduced feed intake implies a reduction in nutrient intake, and, consequently, growth and survivability decline (Teeter and Belay, 1996). Smith and Teeter (1993) showed a 46% decrease in live weight gain of broilers consuming feed *ad libitum* raised at 35^{o}C compared to broilers raised at 23.9^{o}C between 4 and 7 weeks of age. Continuous exposure to elevated temperatures has been shown to reduce immune responsiveness (Thaxton, 1978) while the phagocytic potential of chicken macrophages was also decreased during *in vitro* heat stress conditions (Miller and Qureshi, 1991).

Under environmental conditions that lead to heat distress, the availability of manganese and other minerals is reduced due to increased excretion (Belay and Teeter, 1991). Broilers raised at high cycling ambient temperature (diurnally cycling 24 to 35^{o}C) had lower rates of phosphorus, potassium, sodium, magnesium, sulphur, manganese, copper, and zinc retention compared with birds housed at 24^{o}C (Belay and Teeter, 1996). This lower retention associated with increased urinary excretion of phosphorus, potassium, magnesium, and sulphur, and increased fecal excretion of manganese and copper.

It is possible that the addition of commercial phytase enzyme, or mixed enzymes that contain both phytase and xylanase activity, to rice bran diets, may improve the nutritional value of rice bran, especially in heat-distressed broilers. Also, phytase

enzyme supplementation should have a positive impact by reducing the level of phosphorus excretion and improving the biological availability of phosphorus broilers.

Research Objectives

The project was designed to:

- Evaluate the production performance of broilers fed defatted rice bran diets treated with commercial enzyme phytase alone or a combination of phytase and xylanase under two different environmental temperatures.

- Evaluate the benefit of using commercial enzyme phytase alone or a combination of phytase and xylanase to reduce phosphorus excretion and increase phosphorus availability in broiler fed defatted rice bran diets.

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PART II

LITERATURE REVIEW

Rice Bran

Rice bran is a major by-product from the rice milling process. The paddy or rough rice is the caryopsis of rice harvested with the hull or husk attached. About 20% of paddy rice is hull. The kernel remaining after the hull is removed is brown rice, which has the same gross structure as that of the other cereals. Brown rice consists of about 2% pericarp, 5% seed coat and aleurone, 2-3% germ, and 89-94% endosperm (Hoseney, 1994). Bran is the by-product after removing the outer layers of the pericarp from brown rice to whiten the kernel. The polish or white bran is obtained in the last stages of whitening, mostly consisting of starchy endosperm, aleurone, tegmen, and crushed germ. White bran and bran are usually mixed as a commercially single by-product under the general term "bran", which may contain up to 20% of polish (Juliano, 1972). The amount of bran varies widely, depending on the rice milling procedure, from 4-11% by weight for rough rice and from 5-13.5% by weight for brown rice (Barber and Benedito de Barber, 1985). Rice bran may contain 20-25% total protein, 80% oil, more than 70% minerals and vitamins, and up to 10% starch endosperm of the brown rice (Houston, 1972). Variation in the chemical composition of rice bran may occur due to variety of rice and nature of the milling process. The general proximate analysis of rice bran and polish is shown in Table 1.

The profile of some essential amino acids of rice bran, as shown in Table 2, indicates a good balance and compares favorably with other cereal bran, but none is present in high amount. Rice bran ash is high in phosphorus, potassium, and magnesium. However, the calcium:phosphorus ratio is very low. Rice bran is also an excellent source

Table 1. Proximate analysis of rice bran and polish (dry basis).[1]

Composition (%)	Rice bran	Polish
Protein	9.8-15.4	12.04
Fat	7.7-22.4	16.56
Ash	7.1-20.6	7.97
Nitrogen-free extract	34.2-46.1	56.24
Crude fiber	5.7-20.9	7.33
Pentosans	8.7-11.4	-
Cellulose	5.0-12.3	-

[1]Adapted from Houston (1972).

Table 2. Amino acids content of rice bran.[1]

Amino acid (%)	Australian rice bran	South East Asian rice bran
Arginine	1.23-1.59	1.16
Isoleucine	0.51-0.66	0.51
Leucine	1.01-1.17	1.03
Lysine	0.82-0.91	0.82
Methionine	0.24-0.43	0.28
Phenylalanine	0.50-0.76	0.65
Threonine	0.53-0.64	0.58
Tyrosine	0.51-0.59	0.46
Valine	0.76-1.14	0.77

[1]Adapted from Farrell (1994).

of B-vitamins and vitamin E but contains little or no vitamin A, C, and D. The amount of minerals and vitamins in rice bran is shown in Table 3.

Rice bran is an excellent source of nutrients in animal feeds. However, its high fat content is one disadvantage when this ingredient is included in the diets of animals. If beef and dairy cattle feed contain more than 30% of rice bran, it tends to produce soft-fat and fatty meat or soft or greasy butter (Morrison, 1959). The fat in bran contains a high concentration of unsaturated fatty acids (80-85%), which become rapidly hydrolyzed and oxidized when it is stored without inactivation of lipase (Hoseney, 1994). Fatty acid composition of rice bran oil is shown in Table 4. Hydrolytic change normally occurs in rice bran produced and stored under normal condition. The free fatty acid content of oil increases as a function of time and increases more rapidly with higher moisture content. Up to 50% of the fat in the rice bran was affected within 6 weeks after milling (Warren and Farrell, 1990a). Oxidative deterioration causes the bran to become rancid and unpalatable because of undesirable odors and tastes (Barber and Benedito de Barber, 1985). Hussein and Kratzer (1982) reported no difference in the metabolizable energy between fresh and rancid rice bran measured in adult cockerels. However, the growth rate of chicks given diets containing 60% of rancid rice bran was shown to decrease by more than 18% compared to that of chicks fed diets containing 60% of fresh rice bran.

Rice bran oil can be solvent-extracted from the bran and it is comparable to other edible oils after refining. Animal and human studies have shown cholesterol-lowering from a hypercholesterolemic status with full fat rice bran, with hypocholesterolemic

Table 3. Minerals and vitamins content of rice bran.

Composition	Rice bran
Minerals[1]	
Calcium (%)	0.027-0.051
Phosphorus (%)	1.62-1.81
Magnesium (%)	0.61-0.77
Zinc (μg/g)	44.2-53.9
Iron (μg/g)	37.9-48.1
Vitamins[2]	
Thiamine (μg/g)	10.6
Riboflavin (μg/g)	5.7
Niacin (μg/g)	309.0
Pyridoxine (μg/g)	19.2

[1]Adapted from Warren and Farrell (1990c)
[2]Adapted from Houston (1972).

Table 4. Fatty acid composition of rice bran oil.[1]

Fatty acid (%)	Rice bran oil
Myristic (C14:0)	0.2
Palmitic (C16:0)	15.0
Stearic (C18:0)	1.9
Oleic (C18:1)	42.5
Linoleic (C18:2)	39.1
Linolenic (C18:3)	1.1
Arachidic (C20:0)	0.5
Behenic (C22:0)	0.2

[1]Data from Riceland Foods, Inc.

activity associated with rice bran oil (Kahlon and Chow, 2001). Extraction of rice bran should occur shortly after milling in order to produce stable defatted rice bran. In Thailand, the preparation process of rice bran before sending it into the extractor by heating up rice bran to 110-120^{o}C with steam, can stop the hydrolysis reaction of the oil (www.thaiedibleoil.com). This procedure results in the production of good quality crude rice bran oil and defatted rice bran. Since the starch in defatted rice bran is cooked, it is suitable to be used as a feed ingredient. Randall et al. (1985) also reported that the extrusion cooking of rice bran as soon as possible after milling resulted in the oil that could be stabilized for 30-60 days without an appreciable increase in free fatty acids.

Defatted rice bran is slightly lighter in color and is considerably more bulky than ordinary bran, which has a bulk density of 36.8-40.0 g/100 ml (Houston, 1972). Regular rice bran is a better binding agent in animal feed compounds than defatted rice bran that tends to be quite dusty to handle. Defatted rice bran still contains 0.5-2.5% residual lipids (Lynn, 1969). Metabolizable energy of defatted rice bran is about 75% of that of the full fat rice bran (Ravindran and Blair, 1991). The nutritional value of defatted rice bran is generally equal to the value of full fat rice bran in chicken diets when oil is added to equalize for metabolizable energy (Farrell, 1994).

Some variation in maximum inclusion of rice bran in poultry diets has been reported in published experiments. Rice bran that came from different rice cultivars caused the wide variations in metabolizable energy values. The inclusion of different type of protein (plant origin versus animal protein) in rice bran diets affected the performance of the birds as well (Farrell, 1994). In the review of Farrell (1994), rice bran

can be used up to 20% in chicken diets without or with a small depression in performances. Layers and duckings can tolerate higher level of rice bran in their diets.

Full fat rice bran can be used up to 45% in layer diets (Majun and Payne, 1977; Din et al., 1979; Balnave, 1982). Egg production, shell thickness, and yolk color were adversely affected when diet consisting of 60% rice bran was fed the layers (Majun and Payne, 1977). Egg weight tended to increase in diets containing full fat rice bran because of high level of linoleic acid (Balnave, 1982). Lodhi and Ichhponani (1975) used rice polish compared to defatted rice bran at 20 and 40% in layer diets. They found a reduction in egg size and egg mass in layer fed defatted rice bran diets with no effect on laying performance.

Zombade et al. (1982) determined the feeding value of raw and parboiled rice bran for broilers. They found that parboiled rice bran, which was the by-product from the milling of paddy rice that was subjected to steam treatment and dried before milling, promoted growth of broilers when added at 15% in diet. At 30 and 45% inclusion, both raw and parboiled rice bran adversely affected the performance of the birds. Tan et al. (2000) added lipase-based enzyme preparation to full fat rice bran diets of broilers. Two sources of full fat rice bran were used, one was from Australia (9% inclusion) and another one was from Thailand (9 and 18% inclusion). The enzyme enhanced the apparent metabolizable energy content of full fat rice bran diets but did not increase fat digestibility. Improvements in bird growth were seen only in birds fed full fat rice bran from Thailand. They summarized that the response was dependent upon the geographical origin and the level of rice bran in the diet.

Warren and Farrell (1990b) reported that substitution of defatted rice bran at 7-21% in a basal diet improved growth and feed conversion ratio of broilers from 3-13 days of age. When all diets were balanced to be equal in nutrients, growth rate and feed conversion ratio differed from the controls according to inclusion rate. Farrell and Martin (1998a) reported that there was a significant decline in growth rate and food intake of chicks with increasing rice bran inclusion (0, 20, and 40%, respectively). However, these levels of rice bran did not alter growth rate, feed intake, or feed conversion ratio of duckling at 3-17 day of age. In diets of 19-35 day old ducks, 30% rice bran inclusion stimulated growth, while 60% rice bran inclusion depressed growth but not food intake.

Heat treatment before solvent extraction of rice bran inactivates some antinutritional factors of rice bran harmful to chickens such as trypsin inhibitors (Barber and Benedito de Barber, 1985; Juliano, 1985), however, defatted rice bran still contains high amounts of phytate and non-starch polysaccharides. These are considered to be two major antinutritional factors that limit the use of rice bran in poultry diets.

Phytate

About 70-75% of the total phosphorus in cereals occurs as phytic acid or phytate phosphorus (Hoseney, 1994). It is an important constituent of the outer layers of cereal grain. The currently accepted name of phytic acid is *myo*-inositol 1,2,3,4,5,6 hexakis dihydrogen phosphate or *myo*-inositol hexakisphosphate (IP_6), and phytic acid salts are generally referred to as "phytate(s)" (Sathe and Reddy, 2002). The chemical structure of

phytic acid is shown in Figure 1. Since phytic acid and its salt(s) usually occur simultaneously in many seeds, researchers often refer to them as "phytates". Phytate in plant seeds is predominantly present as the calcium-magnesium-potassium salt called "phytin" (Swick and Ivey, 1992). The amount of phytate varies from 0.06-2.22% in cereals and 0.08-6% in cereal-milled fractions and protein products (Reddy, 2002). In rice, most of phytate is deposited in the germ and pericarp (Reddy et al., 1989). At least 80% of the phosphorus content of brown rice and 40% of the phosphorus in milled rice is phytate phosphorus (McCall et al., 1953). In a most recent report, Reddy (2002) showed that polished rice contains the lowest amounts (<0.25%) of phytate, among all cereals. Rice bran has the highest phosphorus content among cereal brans, of which about 90% is in the form of phytate (McCall et al., 1953, Juliano, 1985). Australian rice bran was found to contain phytate at levels of 2.3-4.0% (Warren and Farrell, 1991).

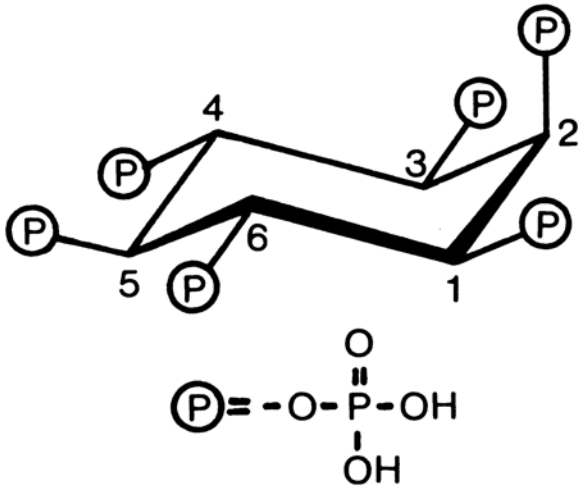


Figure 1. Structure of phytic acid. (Adapted from Hoseney, 1994)

Phytate can be converted to inositol and free phosphoric acid by the enzyme phytase. Almost all plants contain some phytase activity, though the level and the significance of the enzyme in hydrolyzing phytate within the seed varies considerably between plants (Maenz, 2001). Phytate is unavailable to simple-stomach animals because of lack of the enzyme phytase in the gastrointestinal tract (Scott et al., 1982). However, Maenz and Classen (1998) found a specific phytase activity in the brush border membrane of the chick small intestine but the contribution of this enzyme to phytate phosphorus utilization is not known. The undigested phytate in manure can result in serious environmental problem in areas of intensive animal production since the manure from animals is typically applied as fertilizer to agricultural field. It could cause elevated soil phosphorus, phosphorus runoff into streams, and decreased water quality because phosphorus is the limiting nutrient for aquatic plant growth (Sharpley et al., 1994).

Phytic acid is a strong chelator of divalent minerals such as copper, calcium, magnesium, zinc, iron (Fe^2 and Fe^{3+}), cobalt and manganese (Sathe and Reddy, 2002). Because phytate is a polyanionic molecule with six phosphate groups, it can readily form insoluble complexes with divalent cations in weak acidic to neutral pH conditions and, consequently, reduces their bioavailability in animals (Davies and Nightingale, 1975). Zn^{2+} can form the most stable complexes with phytic acid followed by Cu^{2+}, Ni^{2+}, Co^{2+}, Mn^{2+}, Ca^{2+}, and Fe^{2+} in decreasing order of stability (Cheryan et al., 1983). Phytates can also react directly or indirectly with charged groups of proteins, amino acids, or form hydrogen bonds with starch, which may result in an adverse influence on protein and starch digestibility (Sathe and Reddy, 2002). Not only protein in the phytate-bound form

may be less susceptible to protease activity, the formation of less reactive phytate-enzyme complexes can occur because of mineral chelation and thus removal of cofactors required for optimum enzyme activity (Maenz, 2001). The possible bonds of phytase are shown in Figure 2.

Adrizal et al. (1996) reported a decrease in percentage tibia ash of broiler from 4-35 day of age as the level of defatted rice bran increased in the diets (0, 7.5, 15 and 22.5%), whereas body weight and feed conversion ratio were not affected. Nelson and Kirby (1988) suggested that the reduction in tibia ash might be related to an adverse effect of phytate on calcium utilization. Deolankar and Singh (1979) demonstrated a lower distribution of calcium and iron in various organs in broilers fed rice bran compared with corn-based diets due to a reduced absorption of these elements.

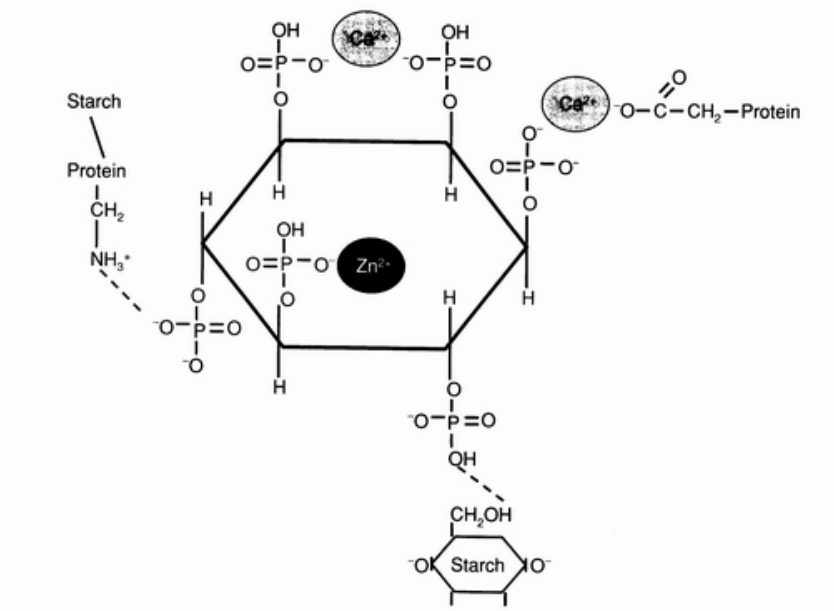


Figure 2. The possible bonds of phytate. (Adapted from Kornegay, 2001)

Thompson and Weber (1981) also found that chicks fed diets containing rice bran had lower zinc content of the tibia. In growing chickens and adult cockerels, rice bran increased the excretion of calcium significantly and of phosphorus and magnesium non-significantly, when added in increment amounts of 0 to 50% (Warren and Farrell, 1991). Warren and Farrell (1990c) reported an occurrence of osteoporosis in laying hens fed diets with 25% defatted rice bran with adequate concentrations of minerals for 6 weeks. In ducks, Farrell and Martin (1998b) found tibia ash declined with increasing rice bran inclusion in diets. Zinc and manganese in ash tended to decline while magnesium tended to increase.

Non-Starch Polysaccharides

Plant materials used as feed ingredients are mostly rich sources of carbohydrates namely sugars, starch, and non-starch polysaccharides. In general, as non-starch polysaccharide level increases across grains, relative metabolizable energy decreases (Grimes and Crouch, 1997). Cellulose and hemicellulose are the major plant structural polysaccharides accounting for approximately 70% of plant biomass (Ladisch et al., 1983).

Cellulose

Cellulose is a linear polymer composed of D-glucose linked by β-1,4 glycosidic bonds. The high degree of order and insolubility, together with its β-linkage, makes it resistant to many organisms and enzymes. Cereals that are harvested with their hulls

intact such as rice, barley, and oats contain more cellulose. Up to 30% of cellulose is present in the pericarp of cereals with only 0.3% or less of cellulose content found in the endosperm (Hoseney, 1994). The distribution of cellulose in brown rice is 62% in bran, 4% in germ, 7% in polish, and 27% in milled rice. The high cellulose in bran is due to the thick cell wall of pericarp, seed coat, and aleurone layer (Juliano, 1972). Certain microorganisms present in the rumen of cattle and in the ceca of horses and rabbits are capable of synthesizing cellulases, which can break down into the disaccharide cellobiose (Leeson and Summers, 2001). Chicken does not possess cellulase in its digestive tract (Scott et al., 1982). Since cellulose is insoluble in water, the possibility of cellulose fermentation in ceca of chicken is very low because only fluid or very small particles are allowed entrance to the ceca (Denbow, 2000).

Hemicellulose

The terms "hemicelluloses" refers to non-starchy and non-cellulosic polysaccharides of plants (Hoseney, 1994). They are the components of plants that are insoluble in boiling water, soluble in dilute alkali, and readily degraded by dilute acid (Scott et. al, 1982). It is the second most abundant plant structural polysaccharide that is present in association with cellulose in plant cell walls (Bhat and Hazlewood, 2001). The chemical composition and structure of hemicelluloses differ widely from a simple sugar, such as that found in β-glucans, to polymers that may contain pentoses, hexoses, proteins, and phenolics. Sugars that are the structural units of cereal hemicellulose include D-xylose, L-arabinose, D-galactose, D-glucose, D-glucuronic acid, and 4-O-methyl-D-glucuronic acid.

The common properties of these hemicelluloses are their resistance to animal digestive enzymes (Hoseney, 1994). Chickens may derive some energy from the hemicelluloses under the acid conditions in the proventriculus and gizzard (Scott et. al., 1982). Therefore, most digestion of the hemicelluloses in poultry occurs in the lower gut by enzymes secreted by resident microflora. The cecum is the main location for microbial degradation of dietary fiber and for absorption of the fermentation products in chicken (Thomas and Skadhauge, 1988). The end products of microbial degradation are various gases (H_2, CO_2, CH_4), lactic acid, and short-chain fatty acids (Jorgensen et al., 1996). The short chain fatty acids have been found to be utilized by birds (Savory, 1992), however, it is not efficient compared with a direct absorption of glucose released from enzymatic digestion (Carre et al., 1995). β-glucans have been found to be least digestible over the entire length of the gastrointestinal tract and arabinoxylans are intermediate in digestibility (Iji, 1999). The structure of two common hemicelluloses is shown in Figure 3.

The solubility in water is another important variation of hemicelluloses (Hoseney, 1994). The cereal hemicelluloses, especially the water-soluble hemicelluloses, as well as some other polysaccharides have a tendency to create a viscous environment in the digestive tract. The viscosity of polysaccharides may have an adverse effect on the digestion of some dietary components and excretion of sticky droppings (Iji, 1999). It is possible that a viscous environment in the small intestine would reduce nutrient mobility and restrict the access of enzymes, especially membrane-bound enzymes, to their dietary substrates. A reduction in the activity of some brush border enzymes, such as alkaline

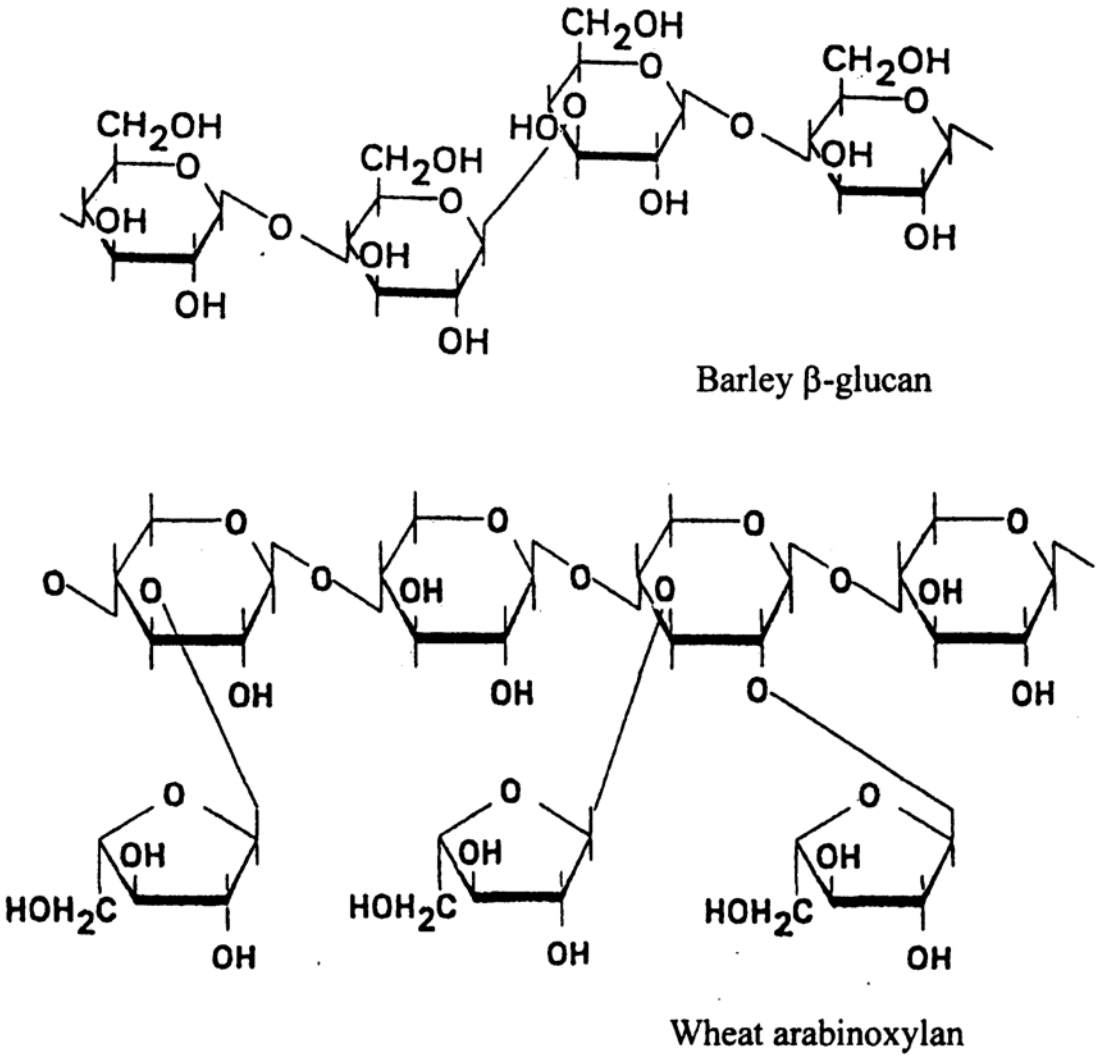


Figure 3. Structure of common non-starch polysaccharides.
(Adapted from Leeson and Summers, 2001)

phosphatase and lactase has also been reported from a study in rats fed semi-synthetic diet containing either guar gum or carboxymethylcellulose (Johnson and Gee, 1986). In an *in vitro* study, Ikeda and Kusano (1983) observed an inhibition of trypsin, α-chymotrypsin, and α-amylase by different indigestible polysaccharides. From the study of Smits et al. (1998), the inclusion of a high viscosity, water-soluble carboxymethylcellulose in diets of broilers impaired the digestibility of lipids and was linked to the reduction in the concentration of bile acids that aid the digestion of lipids. An *in vitro* study of Pasquier et al. (1996) has shown that high viscosity reduced emulsification of dietary lipids and significantly reduced triacylglycerol lipolysis. The size of emulsified droplets was found to be increased and this may slow their rate of diffusion (McCleary et al., 1981). Due to accumulation of undigested material, increased bacterial activity was found in duodenum and jejunum of broilers. It also increased the weight of small intestine and colon of the birds (Smits et al., 1997). The length and weight of the cecum increased with the fiber level (Jorgensen et al., 1996). This may be an indication of changes within the intestinal mucosa, mainly changes in cell proliferation (Iji, 1999). Johnson and Gee (1986) found increased rate of production of mucosal cells in the small and large intestines of rats fed semi-synthetic diet containing either guar gum or carboxymethylcellulose. This increased proliferation was associated with a significant lengthening of the crypts and about 25% increase in the basal width of the villi. The wet weight of the cecal tissue of rats increased nearly fourfold in the rats fed diet containing 10% carboxymethylcellulose compared with that of rats fed no soluble non-starch polysaccharide.

β-*glucans*

β-glucan is a linear polymer composed of D-glucose. It is different from cellulose because the glucose molecules are mixed-linked by β-1,3 and β-1,4 glycosidic bonds (Aman and Westerlund, 1996). The higher solubility of mixed-linked polymer vs cellulose is due to the presence of (1→3)-β-bonds (Clarke and Stone, 1963). Grains are primary source of β-glucans. Some grains have relatively high β-glucan content such as barley (2.0-9.0%), oats (2.5-6.6%), and rye (1%). Other grains have lesser amounts of β-glucans such as wheat (0.5-1.5%), triticale (0.3-1.2%), sorghum (1%), rice (0.6%), and corn (0.1%) (Cho et al., 1997). The soluble β-glucans abundant in oat bran have been shown to have cholesterol-lowering effects in rats and humans (Anderson and Chen, 1986). It has been suggested that the viscous conditions within the small intestine caused by soluble mixed-linked β-glucans of oat and barley is one of the mechanisms involved in hypocholesterolemic responses in humans and animals (Bengtsson et al., 1990; Anderson and Bridges, 1993).

Most water-soluble β-glucans contain approximately 30% (1→3)-, and 70% (1→4)-linkages, which are organized into blocks of two or three (1→4)-linked residues separated by single (1→3)-linked residues (Clarke and Stone, 1963). Oat bran has the highest water-binding capacity compared with barley bran and wheat bran (Wood, 1993). The highly viscous β-glucans in barley and oat limits the bioavailability of these grains when used in animal diets. Young chicks are more affected by barley than adult cocks because the mature birds have a sufficiently developed gastrointestinal tract to overcome

the negative effects of β-glucans (Salih et al., 1991; Almirall and Esteve-Garcia, 1994). Hens fed 40 and 50% barley diets were found to lose weight, however egg production was not affected except yolk color was reduced (Benabdeljelil and Arbaoui, 1994).

Pentosans

Pentosans are various polysaccharides that yield only pentoses on hydrolysis. The pentosans are classified on the basis of water solubility; water-soluble and water-insoluble pentosans that can be extracted with alkali (Autio, 1996). Bedford and Classen (1992) reported a positive correlation between high molecular weight (>500,000) carbohydrate complexes and viscosity in the intestine of chickens fed diets varying in pentosan content. The total and water-soluble pentosans make up about 7-8% and 2-3% of rye grain (Henry, 1987) and 1.4-2.1% and 0.54-0.68% of wheat grain, respectively (Izydorczyk et al., 1991a). Pentosan content of barley varies between 4-8%, and the pentosans are mostly insoluble in water and largely (75%) present in husk (Henry, 1987). The pentosan content of oat and rice varies between 2.2-4.1% and 1.2-4.0%, respectively (Wood, 1992).

Wheat pentosans consist of soluble and insoluble linear arabinoxylans and branched arabinogalactans. The structure of the water-insoluble pentosans is similar to the water-soluble pentosans but with a higher degree of branching (Hoseney, 1994). Pettersson and Aman (1989) reported poor fermentation of insoluble pentosans in cecum and colon of poultry. They suggested that some degradation of insoluble pentosans occurred in the crop and gizzard, while other experiments found insoluble pentosans were

almost completely undigested (Carre et al., 1990; Annison, 1991). The soluble arabinoxylans are responsible for the high viscosity of their solutions in water (Izydorczyk et al., 1991b). However, both the soluble and insoluble components have excellent water-holding capacity. The water uptake capability of pentosans is about 15 gram of water per gram dry basis (Bushuk, 1966). Use of wheat in poultry diets could cause wet litter problem (Bedford and Morgan, 1996). Choct and Annison (1992) reported a reduction in feed intake, weight gain, and feed conversion efficiency and increase in digesta viscosity of broiler chickens when pentosan extracted from wheat was used. Water-soluble rye arabinoxylans also cause an increase in digesta viscosity and decrease the absorption of nutrients in the chick (Fengler and Marquardt, 1988). Severe damage to the intestinal villi and mucous membrane of the small intestine of broiler chicks was observed when rye was used up to 80% in diets (Rakowska et al., 1993).

Cellulose and pentosans occur in rather similar amounts in rice bran (Table 1). Defatted rice bran contains a relatively high percentage of non-starch polysaccharides, chiefly arabinoxylans with the sugar units arabinose and xylose being predominant (Annison et al., 1995), while some galactose is present (Houston, 1972). Shibuya and Iwasaki (1985) reported that xyloglucan was also isolated from the crude hemicellulose of rice bran but the amount of β-(1→3),(1→4)-glucan was very small. Although the soluble isolates from wheat and rice bran were composed mainly of arabinoxylans, they produced different extract viscosities of 64 and 1.6 mPa, respectively (Annison et al., 1995). This was associated with the arabinose:xylose ratio. The water-soluble hemicelluloses of rice bran have an arabinose:xylose ratio of 1.8 (Juliano, 1972), while

wheat have the ratio of 0.58 (Annison et al., 1995). Annison et al. (1995) suggested that rice bran xylan backbone carries more arabinose side-chains, whereas wheat xylan main chain facilitates the formation of inter-chain hydrogen bonds. Alkali-soluble bran hemicellulose contains 37% arabinose, 34% xylose, 11% galactose, 8% protein, 9% uronic acid, and small amount of glucose (Gremli and Juliano, 1970).

Use of Enzymes in Poultry Diets

The role of enzymes in animal feed has become increasingly important after the first successful application of β-glucanases to barley-based broiler diets at the end of the 1980's (Ziggers, 1999). Sheppy (2001) summarized the main reasons for using enzymes in animal feed namely; 1) to break down anti-nutritional factors which have not been digested by animal's endogenous enzymes, 2) to increase the availability of starches, proteins, and minerals that are enclosed within fiber-rich cell wall or bound up in a chemical form that animal is unable to digest, and 3) to supplement the enzymes produced by young animals that have inadequate endogenous enzyme production because of the immaturity of their own digestive system. Enzyme addition not only reduces the variation in nutritive value between feedstuffs, it also increases the uniformity of groups of animals, thus aiding management and improving profitability. Four main types of enzymes currently dominate the animal feed market are enzymes to break down fiber, protein, starch, and phytate. Nowadays, the most often used enzymes in poultry diets are the exogenous fiber degrading enzymes based on xylanase for wheat based diets, β-glucanase for barley based diets, and phytase (Ziggers, 1999). The use of feed enzymes

in the United States of America, except phytase, is not as much as in Europe, Canada or Asia because in the United States broiler diets are corn-soy based. Although the commercial feed enzymes have some basic activities in common, most enzymes are not identical because of the process of production, sensitivity, or side activity. The activity of enzymes measured in a laboratory also has a weak relationship with the effect of those enzymes on animal performance. The commercially mixed feed enzymes are widely available in the market nowadays.

Phytase

Phytase is an esterase that hydrolyzes phytic acid. It catalyzes the removal of orthophosphate from phytate and other *myo*-inositol phosphates (Reddy et al., 1989). One unit of phytase activity is the amount of enzyme that liberates 1 μmol of inorganic phosphorus per minute from 5.1 mmol solution of sodium phytate at 37^{o}C and pH 5.5 (Kornegay, 2001). At least three abbreviations are used for unit of phytase activity: FTU, PU, and U. The degradation of phytate is of nutritional importance because the mineral binding strength decreases and the solubility increases, resulting in an increased bioavailability of essential dietary minerals (Lonnerdal et al., 1989). The study of Zacharias et al. (2003) in growing pigs fed diets based on barley and soybean meal found that the addition of microbial phytase caused an increase in zinc content of the bone and a slight increase in copper requirement. They concluded that it was due to the releasing of zinc from phytate complex and forthcoming antagonistic effect of zinc on copper availability.

Commercial phytase products can be produced by a variety of microorganisms. Currently, these commercial phytases are based on the phytase encoding gene originating from *Aspergillus niger* (Maenz, 2001). The 3-phytases are produced by fungi, while 6-phytases are commonly found in plants (Dvorakova, 1998) with the high phytase activity found in wheat and barley significantly contributing to phytate digestibility (Maenz, 2001). This designation refers to the site of initial hydrolysis on the phytate molecule. No single enzyme is capable of fully dephosphorylating phytate. The efficiency of this hydrolytic process depends upon total phytase activity in combination with other factors, such as the form of phytate within the plant ingredient, amount of minerals in animal diets, pH and retention time in the stomach, and physical condition of the digesta especially in the small and large intestine (Maenz, 2001). Qian et al. (1995) evaluated the influence of calcium:total phosphorus ratio (1.1, 1.4, 1.7, and 2.0:1, respectively) in broiler corn-soybean based diets supplemented with microbial phytase. Added phytase significantly increased body weight gain, feed intake, toe ash content, and phosphorus and calcium retention. These measurements were negatively influenced by widening the dietary calcium:total phosphorus ratio and synergistically improved by addition of vitamin D_3. They suggested that ratio between 1.1:1 to 1.4:1 appeared critical for the efficient use of supplemental phytase and vitamin D_3 in broiler diets.

Leske and Coon (1999) conducted 5-day bioassay to determine effect of phytase on phytate phosphorus hydrolysis and total phosphorus retention of different feed ingredients, included as the sole source of phosphorus, in broilers and laying hens. In 22-day-old broilers without addition of phytase, phytate phosphorus hydrolysis of corn,

soybean meal, wheat, barley and defatted rice bran were 30.8, 34.9, 30.7, 32.2, and 33.2%, respectively. The addition of phytase (600 units/ kg diet) significantly increased each value to 59.0, 72.4, 46.8, 71.3, and 48.0%, respectively. The addition of phytase increased total phosphorus retention from 34.8, 27.0, 16.0, 40.3, and 15.5% to 40.9, 58.0, 33.8, 55.5, and 26.5%, respectively. In laying hens without phytase addition, phytate phosphorus hydrolysis of corn, soybean meal, and defatted rice bran were 23.0, 25.7, and 36.1%, respectively. With addition of phytase (300 units/ kg diet) each value significantly increased to 52.0, 62.4, and 50.9%, respectively. Total phosphorus retention increased from 28.6, 36.8, and 35.9% to 44.7, 53.4, and 43.0%, respectively, with the addition of phytase.

Ravindran et al. (1995) concluded that microbial phytase supplementation generally improves phytate phosphorus utilization in poultry by 20-45% and also improves protein and amino acid digestibility. However, Boling-Frankenbach et al. (2001) found that 1200 units of phytase/kg diet did not significantly increase protein utilization of several feed ingredients as assessed by a protein efficiency ratio (gram weight gain per gram protein intake) chick growth assay. In laying hens fed corn-soybean meal diets containing low level of non-phytate phosphorus (0.10%), supplementation of phytase improved feed intake, feed conversion ratio, egg mass, and elicited a response in shell quality and egg components (Jalal and Scheideler, 2001). Keshavarz (2000) found that phytase (300 units/kg diet) supplementation significantly increased phytate phosphorus retention of laying hens by about 15%. Daily total phosphorus excretion was 47% less for 66-week-old hens fed phytase-supplemented diets

containing 0.10% non-phytate phosphorus than for the unsupplemented phytase control group fed 0.30% non-phytate phosphorus in the diet.

Farrell and Martin (1993) reported the result of adding phytase at 1000 units/kg to the duck finishing diets from 19-40 days. Feed conversion ratio was improved at the three levels of rice bran inclusion (0, 30, and 60%). Tibia ash also showed a response to phytase only with 0 and 30% rice bran inclusion.

β-*glucanase and Xylanase*

One unit of β-glucanase activity (BGU) is the amount of enzyme required to liberate 1 μmol of glucose reducing-sugar equivalents per minute under the conditions of the assay (McCleary, 2001). The addition of a crude enzymatic preparation which contained β-glucanase to a barley-based diet was shown to significantly improve weight gain and feed conversion ratio, and reduce the relative length of the intestinal parts and the caeca of broilers at 28 day of age (Viveros et al., 1994). It also improved the morphological changes of jejunum epithelium (shortening, thickening and atrophy of the villi and an increment in the number and size of globet cells) that occurred in birds fed barley-based diet without enzyme supplementation. Almirall and Esteve-Garcia (1994) studied rate of food passage in the three-week-old broilers and one-year-old Leghorn cocks fed 60% barley-based diets by adding chromium oxide in the diets. Peak chromium oxide excretion occurred 1 hour later in broilers than in cocks. With β-glucanase addition, the age-related difference was removed. The digesta retention time

of broilers decreased and the digesta retention time of cocks increased, and both rates became similar. These results are consistent with the studies of Salih et al. (1991).

Xylanases, in general, are specific for the internal β-1,4 linkages of polymeric xylan and are designated as endoxylanases (Bhat and Hazlewood, 2001). One unit of xylanase activity (XU) is the amount of enzyme required to liberate 1 μmol of xylose reducing-sugar equivalents in one minute under the conditions of the assay (McCleary, 2001). Many researchers have found that adding xylanase to wheat-based diet can reduce the negative effects of wheat non-starch polysaccharide, reduce viscosity in the gut, and consequently, release nutrients trapped by the highly viscous gut content (Grimes and Crouch, 1997). The end result is better digestion and absorption of nutrients from wheat and other feed ingredients. Marron et al. (2001) studied the effects of adding xylanase in wheat-based broiler diets. Enzyme addition did not significantly affect dry matter intake or live weight gain, however, it improved gain:feed, apparent metabolizable energy:gross energy, ileal apparent digestibility of dry matter and starch, and reduced *in vivo* viscosity. However, wheat can vary in non-starch polysaccharide level and poultry seem to be variable in their response to wheat based rations. For the wheat variety where gut viscosity was not affected much, addition of enzyme had little or no effect on apparent metabolizable energy (Grimes and Crouch, 1997).

Supplementation of enzyme preparation having both β-glucanase and arabinoxylanase activities to a rye-wheat-based broiler diets was found to improve weight gain of broilers up to 27% and feed conversion ratio up to 10% (Pettersson and Aman, 1988; 1989). Bedford and Classen (1992) also found improvement in weight gain and

feed conversion ratio of broiler chicks with increasing pentosanase (contained both xylanase and β-glucanase) concentration and decreasing rye concentration in diets. The intestinal viscosity, which rose as digesta passed from the proximal to distal small intestine, also fell with pentosanase addition and decreasing rye concentration. Supplementation of an enzyme complex containing β-glucanase, xylanase, and cellulase activities in laying hen barley-based diets significantly improved feed efficiency and reduced water: feed ratio and incidence of dirty eggs (Francesch et al., 1995).

An attempt to improve the nutritive value of Australian full fat rice bran in chickens and ducks by adding enzyme preparations containing xylanase in diets resulted in no response (Farrell and Martin, 1998a). Relative gut viscosity declined with increased rice bran inclusion (0, 20, and 40%, respectively). They concluded that non-starch polysaccharide in rice bran was not a significant factor in altering the nutritive value of rice bran. However, positive effects of xylanase supplemented rice bran diets were found in the experiments of Wang et al. (1995). There were significant improvements in feed consumption, weight gain, and feed conversion ratio in Leghorn chicks fed wheat based diet containing 40% rice bran and corn-based diet containing 25% rice bran supplemented with xylanase.

Secondary Benefit of Using Enzymes in Poultry Diets

Considerable interest in the use of phytase as a feed additive has been increasing recently, as it not only increases the availability of phosphate in plant feed ingredients but also reduces environmental pollution from the excess phytate phosphorus in manure.

However, controversy exists concerning the use of phytase to improve phosphorus retention and the possibility that water-soluble phosphorus and total phosphorus ratio in broiler litter would be increased. The concern focuses on the runoff from pasture fields that have been fertilized with litter from broilers fed diets supplemented with phytase, especially the diets that contain high level of total phosphorus. The study of Rapp et al. (2001) in minipigs fitted with re-entrant cannulas found that phytase activity in ileal digesta 12 hours after feeding was not significantly different among pigs fed diets with 0, 818, or 1192 units of phytase. Jongbloed et al. (1992) also found negligible phytase activity in the terminal ileum in pigs fed 1500 U phytase/kg diets. From the study of Applegate et al. (2003) in broilers fed different levels of total phosphorus with phytase (600 U/kg), no differences were found in the water-soluble phosphorus concentrations in the litter collected at 49 day of age. Therefore, phytase activity should not affect the water-soluble phosphorus content in the manure of animals fed phytase-supplemented diets. Other factors, such as differences in lower intestinal microflora and differences in litter conditions or litter microorganisms, may initiate the release of inorganic phosphorus and increase water-soluble phosphorus concentrations (DeLaune et al., 2001)

The use of feed ingredients that contain large quantities of soluble, viscous arabinoxylans and β-glucans, cause significantly reduced rate of digestion, consequently providing greater substrate for lower small intestinal and large intestinal/caecal resident flora (Bedford and Apajalahti, 2001). An increased amount of non-starch polysaccharides in the digesta has been found to have a negative influence on the gut microflora of the birds (Choct et al., 1996). Not only is there are increase in the total

bacterial number, but also the profile of the inhabitants is altered (Vahjen et al., 1998). Viscosity presumably favors the growth of such growth-depressing bacteria through its effect on the rate of digestion. Populations of enterococci and coliforms have been observed to increase in chickens fed rye and barley based diets, which affect the performance and health status of the bird. The use of antibiotics in animal feed as a growth promotant has been demonstrated to be a partial solution for this problem (Campbell et al., 1983).

Enzymes enhance the digestion of feed and thus have a stabilizing effect on the composition of gut flora (Ziggers, 1999). Enzymes aid in accelerating digestion at the level of the ileum (Bedford and Schulze, 1998) thereby reducing the available substrate reaching the bacterial populations in the intestine. The general health status of birds can be indirectly improved, resulting in fewer non-specific digestive upsets (Sheppy, 2001). The role of enzymes will become even more important when antibiotics are left out of the feed.

Effect of Heat Stress in Poultry

Heat stress is a problem in poultry production in many regions of the world. Poultry are homeothermic (Hillman et al., 1985). As the ambient temperature and relative humidity rise above the comfort zone, a wide variety of physiological, behavioral, neuroendocrine and molecular responses are initiated to maintain normal body temperature. Several factors such as acclimatization, diet, relative humidity, diurnal

temperature variation, genetics, age, air velocity, and weight may be involve in the capability of poultry to tolerate high environmental temperature (Carr and Carter, 1985). Freeman (1966) reported that the comfort zone or the zone of thermal neutrality in chicks 1-7 days of age is 35^{o}C and from 7-21 days of age is 31^{o}C, respectively. An acceptable brooding ambient temperature at 35^{o}C can cause the heat stress response in birds at 4-week of age (Teeter and Belay, 1996). Maximum growth rate of the 3-7 week-old broilers occurs at approximately 20^{o}C (McNaughton and Reece, 1982). The primary consequence of elevated growing temperature is reduction in feed intake (Dale and Fuller, 1980) in order to reduce metabolic heat production and maintain body temperature within the upper and lower limits of a circadian rhythm in deep body temperature. In well-fed chickens that are neither losing heat to the environment nor gaining heat from the environment, the lower and upper limit of the circadian rhythm is about 40.5-41.5^{o}C (Etches et al., 1998). When ambient temperature rapidly rose above 30^{o}C, body temperature of the chickens began to increase (Boone, 1968). However, if the ambient temperature rose more slowly, the body temperature of the chickens became constant and began to rise when the ambient temperature reaches 33^{o}C (Boone and Hughes, 1971).

Behavioral adjustments can occur rapidly. Chickens consumes less feed and more water to compensate for water lost through evaporative cooling from respiratory surfaces in order to dissipate body heat. The increase in water consumption can occur immediately, whereas the reduction in food consumption will happen several hours after the birds have experienced high temperature (May and Lott, 1992). Smith and Teeter (1993) showed a 46% decrease in live weight gain of broilers consuming feed *ad libitum*

at 35°C compared to broilers raised at 23.9°C between 4 and 7 weeks of age. Most climates have high environmental temperatures during the day and diurnal cycles with cooler nights. The response of chicken to cyclic temperatures appears to depend on the mean temperature and the amplitude of the cycle (Austic, 1985). Food intake may be estimated from the average temperature of the cycle. Food intake of broilers was decreased 20% in the hot cyclic environment (23.9-35°C) compared to normal cyclic environment (12.8-23.9°C) (Keshavarz and Fuller, 1980). The average of 1.8%/°C decline in food intake on the average temperature of the cycles is similar to the study of Harris et al. (1974) when broilers were raised using 18.3-35°C or 23.9-35°C cycle compared to using 18.3-23.9°C cycle. Austic (1985) estimated the change in food intake of broilers about 1.5%/°C as environmental temperature varies from 20°C. In laying hens, the drop in feed consumption was estimated about 1.5%/°C when temperature rise between 21 and 30°C, and 4.6%/°C between 32 and 38°C (Payne, 1966). Belay and Teeter (1991a) found that broilers raised in cycling temperature (25-35°C) had 14.5% mortality. The mortality rate decreased to 6.3% in chickens fed low energy low calorie-protein ratio diet. They summarized that higher calorie-protein ratios have ability to increase broiler growth rate during heat stress, however, broilers would significantly increase fat deposition and mortality risk. Temim et al. (2000) found that chronic heat stress markedly reduced protein synthesis of broilers fed 20 or 25% protein diets, leading to reduce protein deposition. At 32°C, the high protein diet did not significantly modify protein synthesis. However, it favored muscle protein deposition, which was probably related to reduced proteolysis.

Water intake of broilers increased very little as environmental temperature increased from 2, 9 and 16, to 22^{o}C, then increased greatly at 30 and 35^{o}C (Farrell and Swain, 1977). The heat loss due to evaporation of water at 2^{o}C was approximately 11% and reached a maximum of approximately 100% at 35^{o}C. Belay et al. (1991) found the voluntary consumption, water retention, and water excretion increased by 76.4, 59.7, and 144%, respectively, for broilers raised at fixed feed (3.5% body weight) using 24-35^{o}C cycle compared with broilers housed in 24^{o}C environment. When water consumption was fixed at 8.5% body weight, water loss was increased by 63% in broilers using 24-35^{o}C cycle. Panting is the response of poultry during exposure to heat. Respiratory frequency gradually increases with increasing body temperature (Hillman et al., 1985). This specialized form of respiration helps animals to reduce their total heat load by evaporative cooling at the surfaces of the mouth and respiratory passageways (Etches et al., 1998). The rate of evaporation from panting hen increases from 5 to 18 g/hour in response to a change in ambient temperature from 29 to 35^{o}C with relative humidity of 50-60% (Lee et al., 1945). Panting also increases the loss of carbon dioxide from the lungs thereby reducing the partial pressure of carbon dioxide and bicarbonate in blood plasma (Wang et al., 1989). The lower concentration of hydrogen ions causes a rise in plasma pH (Mongin, 1968), generally referred to as alkalosis. Egg shell strength declines markedly at high environmental temperature which is probably result of hyperventilation and the consequent hypobicarbemia (Austic, 1985).

Broilers exposed to a constant 35^{o}C until 6 week of age compared with broilers kept at 24^{o}C had significantly increased urinary mineral excretion (mg/kg body weight)

of K^{+} (57.85, 28.87), Mg^{2+} (6.96, 4.64), P^{3-} (64.63, 41.22), S^{2-} (27.80, 22.32), Ca^{2+} (4.3, 2.0), Na^{+} (10.9, 6.4), Mn^{2+} (0.02, 0.01), and Se^{2-} (0.15, 0.06), respectively (Belay and Teeter, 1991b). No differences were found for urinary loss of Zn^{2+}, Cu^{+}, Fe^{2+}, and Mo^{6+}. Urinary chloride excretion of birds raised at 35°C was significantly lower reflecting their respiratory alkalosis. These results were similar to results from another experiment in the same laboratory (Belay and Teeter, 1996), which also found that broilers exposed to 35°C increased urine output from 50.7 ml/12 hours/kg body weight at 24°C to 101.3 ml/kg body weight. This was associated with an increased urine:water ratio and reduced urine osmolality.

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PART III

EXPERIMENT 1: BROILER PERFORMANCE AND MINERAL UTILIZATION OF PHYTASE-SUPPLEMENTED DEFATTED RICE BRAN DIET DURING HEAT STRESS

Abstract

An experiment was conducted to determine broiler performance and mineral utilization of defatted rice bran diets supplemented with phytase under two different environmental temperatures. Six replicate groups of chicks were assigned to five dietary treatments comprised of a control corn-soybean meal diet with 25% corn starch and four diets containing two levels of defatted rice bran (10 and 25%) with or without a commercial phytase (500 units/kg diet) supplementation. Available phosphorus in diets containing phytase was calculated to be 0.1% reduction from the requirement for broilers. On day 21, some birds were slaughtered and tibias harvested for mineral analysis. The remaining birds were transferred to either a thermoneutral temperature chamber (23.9^{o}C) or a heat stress chamber (23.9-35^{o}C, diurnal). Digesta retention time was determined and a 5-day total excreta collection was done at day 35. Birds were slaughtered at seven weeks of age, tibias were harvested, and intestines examined. Fat added into defatted rice bran diets seemed to promote the growth performance of birds. Using up to 25% defatted rice bran in diets was not detrimental to broilers. The beneficial effect of high fat level could overcome the problem associated with high phytate phosphorus and non-starch polysaccharide in defatted rice bran. Feed cost per weight gain of birds fed 25% defatted rice bran diets was significantly lower. Using phytase in diets did not affect the performance, carcass quality, morphology of the intestine, or feed cost per weight gain of birds. Percent phosphorus, zinc and manganese content in the tibia of chicks fed 10% defatted rice bran diet were improved by supplemental phytase. Although percent phosphorus in excreta was decreased by 10.1% when using phytase in 25% defatted rice

bran diet, phosphorus retention of birds did not improve significantly. Higher levels of phytase might be more beneficial. The adverse effects of heat stress on growth performance could not be improved by phytase supplementation.

Introduction

Rice bran is a major by-product from the rice milling process. The amount of bran by weight of brown rice can vary widely from 5-13.5%, depending on the rice milling process (Barber and Benedito de Barber, 1985). Rice bran is an excellent source of nutrients in animal feed. It contains approximately 9.8-15.4% protein with 7.7-22.4% fat (Houston, 1972). Rice bran oil can be extracted from rice bran. Defatted rice bran can also be used as animal feed ingredient. The nutritional value of defatted rice bran is generally equal to the value of rice bran after the diet is adjusted with fat to equalize for metabolizable energy (Farrell, 1994).

Some of the factors present in natural feed ingredients might have a negative impact on the bioavailability of a trace elements such as manganese are phytic acid, fiber, and calcium. All of these factors are represented in one way or another in the common feed ingredients, such as corn, soybean meal, fish meal, and rice bran (Halpin and Baker, 1986).

Defatted rice bran contains over 700 g /kg of non-starch polysaccharides of which arabinose and xylose are predominant (Annison et al., 1995). High levels of non-starch polysaccharide, especially the water-soluble part, in feed ingredients can be detrimental

to broilers. Defatted rice bran is also high in phosphorus, however about 90% is in the form of phytate phosphorus (Juliano, 1985). Phytate is unavailable to simple stomach animals because of lack of phytase in gastrointestinal tract (Scott et al., 1982). Phytase is an enzyme that converts phytate to inositol and free phosphoric acids. Phytate can form insoluble complexes with many divalent cations in weak acidic to neutral pH conditions. Therefore, the bioavailability of minerals such as zinc, copper, manganese, or calcium, will be decreased in animals fed diets with high levels of phytate (Cheryan et al., 1983). The undigested phytate in animal manure can also cause an environmental problem because of increased soil phosphorus, phosphorus runoff, and decreased water quality (Sharpley et al., 1994).

The use of exogenous phytase as a feed additive has been increasing recently, not only to improve the bioavailability of phosphate and other minerals in plant feed ingredients, but also to reduce the amount of excess phytate phosphorus in the manure. The bioassay to determine effect of phytase on phytate phosphorus hydrolysis and total phosphorus retention done by Leske and Coon (1999) found that the addition of phytase significantly increased phosphorus hydrolysis and total phosphorus retention from defatted rice bran in broiler and laying hen rations. Improvement in protein and amino acid digestibility was also found when using phytase in poultry diets (Ravindran et al., 1995).

Poultry production is adversely affected by heat distress. Its effects are economically significant and geographically widespread. The primary consequence of elevated growing temperature is reduction in feed intake in order to reduce metabolic

heat production which is followed by reduction in growth and survivability of birds (Dale and Fuller, 1980; Teeter and Belay, 1996). The availability of manganese and other minerals is reduced due to increased excretion (Belay and Teeter, 1991). Lower rates of phosphorus, potassium, sodium, magnesium, sulphur, manganese, copper, and zinc retention were found in birds under heat stress condition (Belay and Teeter, 1996). It is possible that the addition of commercial phytase to defatted rice bran diets may improve the nutritional value of defatted rice bran, especially in heat-distressed broilers. Also, phytase supplementation could have a positive impact in reducing the level of phosphorus excretion and improve the biological availability of phosphorus and other minerals in broilers. Therefore, the purposes of this experiment were to:

- Evaluate the production performance of broilers fed defatted rice bran diets supplemented with commercial phytase under two different environmental temperatures.

- Evaluate the benefit of using commercial phytase to reduce total phosphorus excretion and improve bioavailability of phosphorus, calcium, zinc, and manganese of birds fed defatted rice bran diets.

Materials and Methods

Chickens and Diets

The procedures used in the animal work were approved by the University of Tennessee Institutional Animal Care and Use Committee. Four hundred and twenty day-old mixed sex commercial broilers (Ross x Ross) were used in this experiment. They

were distributed among thirty pens in a single brooder house. Six replicate groups of fourteen chicks were randomly assigned to each of five dietary treatments:

Diet 1: control diet with 25% corn starch, without defatted rice bran

Diet 2: 15% corn starch, 10% defatted rice bran

Diet 3: 15% corn starch, 10% defatted rice bran + phytase

Diet 4: 25% defatted rice bran

Diet 5: 25% defatted rice bran + phytase

Composition of defatted rice bran used in this experiment is shown in Table 1. Diets were formulated to meet or exceed current National Research Council recommendations (NRC, 1994), with the control diet being corn-soybean meal based with 25% corn starch (Table 2, 3, and 4). The substitution of corn starch with 10 and 25% defatted rice bran was done in treatment 2-3 and 4-5, respectively. Corn starch contains very low amount of total phosphorus, therefore the increased levels of total phosphorus in the diets were due to the increased phytate phosphorus from defatted rice

Table 1. Composition of defatted rice bran.[1]

Composition	Defatted rice bran
Crude protein, %	16.97
Fat, %	2.23
Ash, %	12.52
Calcium, %	0.95
Total phosphorus, %	2.03
ME, kcal/kg[2]	1700

[1] Defatted rice bran provided by Riceland Foods, Stuttgart, Arkansas
[2] Data from Riceland Foods, Inc.

Table 2. Composition of starter diets (%).

Treatment	1	2	3	4	5
Corn	28.44	30.13	30.61	30.92	31.96
Corn starch	25.00	15.00	15.00	-	-
Defatted rice bran	-	10.00	10.00	25.00	25.00
Soybean meal	21.80	16.82	16.74	11.94	11.54
Corn gluten meal	10.00	10.00	10.00	9.99	9.97
Fish meal	5.93	7.00	7.00	6.80	7.00
Tallow	2.79	5.44	5.27	9.97	9.67
Vitamin Mix[1]	1.00	1.00	1.00	1.00	1.00
Dicalcium phosphate	1.95	1.89	1.35	1.77	1.23
Limestone	0.53	0.12	0.43	-	0.02
Salt	0.30	0.30	0.30	0.30	0.30
L-Lysine	-	0.05	0.05	0.13	0.13
DL-Methionine	0.16	0.15	0.15	0.08	0.08
Salinomycin	0.10	0.10	0.10	0.10	0.10
Sand	2.00	2.00	1.95	2.00	1.95
Phytase[2]	-	-	0.05	-	0.05
Total	100	100	100	100	100
Calculated nutrient composition					
Crude protein, %	23.00	23.00	23.00	23.00	23.00
ME, kcal/kg	3200	3200	3200	3195	3200
Calcium, %	1.00	1.00	1.00	1.10	1.00
Available phosphorus, %	0.45	0.45	0.35	0.45	0.35
Total phosphorus, %	0.81	0.97	0.87	1.17	1.08
Zinc, ppm	106.1	108.2	107.7	109.7	109.4
Manganese, ppm	126.8	147.0	145.4	177.7	176.1
Lysine, %	1.09	1.09	1.09	1.09	1.09
Methionine, %	0.60	0.60	0.60	0.54	0.54

[1] Supplied per kilogram of diet: Copper, 8 mg; Iodine, 0.44 mg; Iron, 100 mg; Manganese, 100 mg; Selenium, 0.3 mg; Zinc, 75 mg; Vitamin A, 8800 IU; Vitamin D_3, 2992 ICU; Vitamin E, 29.92 IU; Vitamin B_{12}, 0.02 mg; Menadione, 1.65 mg; Biotin, 0.2 mg; Choline, 550 mg; Folic acid, 1 mg; Niacin, 66 mg; d-Pantothenic acid, 11 mg; Vitamin B_6, 4.4 mg; Riboflavin, 6.6 mg, Thiamine, 1.1 mg.

[2] Allzyme SSF Phytase, provided by Alltech, Nicholasville, Kentucky, contains 1000 PU/g

Table 3. Composition of grower diets (%).

Treatment	1	2	3	4	5
Corn	36.19	36.80	37.26	36.83	37.27
Corn starch	25.00	15.00	15.00	-	-
Defatted rice bran	-	10.00	10.00	25.00	25.00
Soybean meal	16.44	13.48	13.44	10.40	10.37
Corn gluten meal	10.00	10.00	10.00	8.70	8.64
Fish meal	4.64	4.20	4.16	4.26	4.28
Tallow	1.71	4.74	4.58	9.58	9.43
Vitamin Mix[1]	1.00	1.00	1.00	1.00	1.00
Dicalcium phosphate	1.45	1.36	0.83	1.23	0.70
Limestone	1.03	0.82	1.13	0.39	0.70
Salt	0.30	0.30	0.30	0.30	0.30
L-Lysine	0.14	0.20	0.20	0.21	0.21
Salinomycin	0.10	0.10	0.10	0.10	0.10
Sand	2.00	2.00	1.95	2.00	1.95
Phytase[2]	-	-	0.05	-	0.05
Total	100	100	100	100	100
Calculated nutrient composition					
Crude protein, %	20.32	20.32	20.32	20.46	20.46
ME, kcal/kg	3200	3200	3200	3200	3200
Calcium, %	1.00	1.00	1.00	1.00	1.00
Available phosphorus, %	0.35	0.35	0.25	0.35	0.25
Total phosphorus, %	0.67	0.79	0.69	1.00	0.90
Zinc, ppm	102.1	102.9	102.3	105.2	104.7
Manganese, ppm	123.1	143.6	142.0	175. 0	173.4
Lysine, %	1.00	1.00	1.00	1.00	1.00
Methionine, %	0.40	0.40	0.40	0.40	0.40

[1] Supplied per kilogram of diet: Copper, 8 mg; Iodine, 0.44 mg; Iron, 100 mg; Manganese, 100 mg; Selenium, 0.3 mg; Zinc, 75 mg; Vitamin A, 8800 IU; Vitamin D_3, 2992 ICU; Vitamin E, 29.92 IU; Vitamin B_{12}, 0.02 mg; Menadione, 1.65 mg; Biotin, 0.2 mg; Choline, 550 mg; Folic acid, 1 mg; Niacin, 66 mg; d-Pantothenic acid, 11 mg; Vitamin B_6, 4.4 mg; Riboflavin, 6.6 mg, Thiamine, 1.1 mg.

[2] Allzyme SSF Phytase, provided by Alltech, Nicholasville, Kentucky, contains 1000 PU/g

Table 4. Composition of finisher diets (%).

Treatment	1	2	3	4	5
Corn	41.04	41.12	41.57	40.08	40.587
Corn starch	25.00	15.00	15.00	-	-
Defatted rice bran	-	10.00	10.00	25.00	25.00
Soybean meal	15.82	14.10	14.07	13.46	13.33
Corn gluten meal	8.71	6.27	6.23	2.84	2.79
Fish meal	2.47	3.81	3.81	4.28	4.36
Tallow	1.26	4.61	4.46	9.98	9.81
Vitamin Mix[1]	1.00	1.00	1.00	1.00	1.00
Dicalcium phosphate	1.18	1.10	0.56	0.96	0.42
Limestone	0.96	0.49	0.80	-	0.30
Salt	0.30	0.30	0.30	0.30	0.30
L-Lysine	0.16	0.10	0.10	-	0.003
Salinomycin	0.10	0.10	0.10	0.10	0.10
Sand	2.00	2.00	1.95	2.00	1.95
Phytase[2]	-	-	0.05	-	0.05
Total	100	100	100	100	100
Calculated nutrient composition					
Crude protein, %	18.35	18.35	18.35	18.40	18.40
ME, kcal/kg	3200	3200	3200	3200	3200
Calcium, %	0.80	0.80	0.80	0.80	0.80
Available phosphorus, %	0.30	0.30	0.20	0.30	0.20
Total phosphorus, %	0.56	0.73	0.63	0.95	0.85
Zinc, ppm	98.78	101.9	101.4	105.3	104.9
Manganese, ppm	121.6	143.1	141.5	175.5	173.9
Lysine, %	0.90	0.90	0.90	0.87	0.88
Methionine, %	0.35	0.35	0.35	0.34	0.34

[1] Supplied per kilogram of diet: Copper, 8 mg; Iodine, 0.44 mg; Iron, 100 mg; Manganese, 100 mg; Selenium, 0.3 mg; Zinc, 75 mg; Vitamin A, 8800 IU; Vitamin D_3, 2992 ICU; Vitamin E, 29.92 IU; Vitamin B_{12}, 0.02 mg; Menadione, 1.65 mg; Biotin, 0.2 mg; Choline, 550 mg; Folic acid, 1 mg; Niacin, 66 mg; d-Pantothenic acid, 11 mg; Vitamin B_6, 4.4 mg; Riboflavin, 6.6 mg, Thiamine, 1.1 mg.

[2] Allzyme SSF Phytase, provided by Alltech, Nicholasville, Kentucky, contains 1000 PU/g

bran. Tallow was used as the dietary fat source. Phytase (Allzyme SSF Phytase, Alltech Inc.) containing 1000 units of phytase per gram, was used at 0.05% in the diet. The level of available phosphorus in all diets containing phytase was reduced by 0.10%: from 0.45% to 0.35% in starter period (0-3 week of age), 0.35% to 0.25% in grower period (3-6 week of age), and 0.30% to 0.20% in finisher period (6-7 week of age), respectively. Analyzed nutrient composition of the experimental diets is shown in Table 5. All diets were fed in mash form. Chicks were weighed and allowed *ad libitum* access to feed and water. During the first 21 days posthatch, birds were kept under 23 hours light:1 hour dark photoperiod and thermoneutral condition. Weekly feed consumption and body weight were recorded.

Temperature Treatments

On day 22, eight birds from each replicate were randomly selected and weighed individually. They were divided into two groups and transferred to individual metabolism cages in two environmental chambers, maintaining the same dietary treatments for the next four weeks (Table 3 and 4). The ambient temperature in one chamber was controlled to be constant at 23.9^{o}C (thermoneutral) while the other one was allowed to cycle between 23.9 and 35^{o}C (heat stress) in order to simulate diurnal temperature pattern. The photoperiod was held at 23 hours light:1 hour dark for both chambers. Feed consumption, water consumption, and body weight were monitored on a weekly basis. The remaining birds were humanely slaughtered and both tibias of three birds from each replicate were harvested and frozen for calcium, phosphorus, zinc, and manganese analysis.

Table 5. Analyzed nutrient composition of experimental diets (%).

Starter diets	Treatment				
	1	2	3	4	5
Crude protein, %	28.30	27.99	28.25	28.15	26.96
Fat, %	3.85	7.37	7.19	12.66	12.49
Ash, %	8.65	10.43	10.69	11.44	10.15
Calcium, %	0.99	1.13	1.25	1.03	0.98
Total phosphorus, %	0.99	1.13	1.10	1.22	1.15
Grower diets	Treatment				
	1	2	3	4	5
Crude protein, %	25.08	23.98	24.72	24.72	24.10
Fat, %	2.54	5.87	6.71	12.03	11.53
Ash, %	8.47	8.98	8.63	9.57	9.87
Calcium, %	1.10	1.18	1.14	0.95	0.89
Total phosphorus, %	0.67	0.87	0.80	1.17	1.00
Finisher diets	Treatment				
	1	2	3	4	5
Crude protein, %	21.65	22.59	21.47	21.43	21.51
Fat, %	2.15	6.60	6.73	13.07	13.17
Ash, %	7.48	9.30	9.22	10.10	9.30
Calcium, %	0.86	0.89	0.79	0.82	0.80
Total phosphorus, %	0.55	0.66	0.63	1.03	1.00

Digesta Retention Time Measurement and Excreta Collection

On day 35, following a 12-hour fast, birds were allowed to consume 30 g ferric oxide labeled feed (1.5% ferric oxide) as an indigestible marker following which the time of first appearance of a bright red tint of ferric oxide in the feces was noted as a measure of digesta retention time. Two male and two female birds from each treatment within each chamber were monitored for excreta collection. Individual stainless steel trays were placed under each cage for excreta collection. The amount of feed intake and excreta were recorded and all of the excreta was collected for 5 consecutive days. Ferric oxide labeled feed was again given on the fifth day and excreta collection ceased when a bright red tint of ferric oxide appeared following the second dosing. The collected excreta was frozen for analysis of calcium and phosphorus and apparent metabolizable energy determination.

Processing of Birds

On day 49, following a 12-hour fast, five male and five female birds from each treatment within each chamber were humanely slaughtered. The carcasses were then manually eviscerated. The whole intestines were carefully pulled out. Abdominal fat pad (including fat surrounding gizzard, bursa of Fabricius, cloaca, and adjacent muscles) was weighed individually and calculated as a percentage of live weight of the bird. Following evisceration, each part of intestine of two male and two female birds from each treatment within each chamber were separated; the intestinal segment from the gizzard to pancreatic and bile ducts as duodenum, jejunum from the ducts to Meckel's

diverticulum, and ileum from the diverticulum to the ileo-cecal-colonic junction. After removal of residual digesta by manual compression, the weights of duodenum, jejunum, ileum, ceca, and colon were determined. Each part of intestine was calculated as a percentage of the whole intestinal weight, and the whole intestinal weight was calculated as a percentage of live weight of the bird. Duodenum and jejunum of the rest of the birds were carefully collected for villi height and crypt depth measurements. Each carcass was weighed, separated into market portions (breast, thigh, legs, wings, back) and each part weighed. The parts yield was calculated as a percentage of the whole carcass. During the slaughtering process, the right tibia was harvested and frozen for calcium, phosphorus, zinc, and manganese analysis.

Villi Height and Crypt Depth Measurements

The duodenum and jejunum of three male and three female birds from each treatment within each chamber collected during the slaughtering process were carefully separated. The middle part of each duodenum and jejunum segment was cut about 6 centimeters long. Each portion was cut again half way along the side, opened up, and rinsed lightly inside and outside with buffered formalin. Each sample was stored at room temperature in 30 ml buffered formalin. Later, slide sections were prepared by paraffin embedding the sample, sectioning, and staining with hematoxylin-eosin.

The slide of each sample was studied under an automated microscope. The photographs of at least 10 villi per slide, showing complete section of villi and crypt, were taken by a digital camera connected to a computerized MetaMorph Imaging

System. The system measured villi height and crypt depth by calculating a distance (micron) along the line from the villus tip to the bottom and from the bottom of the villus to the deepest part of crypt, respectively. The example of villi height measurement is shown in Figure 1. The mean villi height and crypt depth of duodenum and jejunum of each bird were used for statistical analysis.

Tibia Mineral Determination

Tibias of birds harvested at 3-week and 7-week of age were prepared for calcium, phosphorus, zinc, and manganese analysis. All adhering tissue was removed from the bones. Two tibias from the same 3-week-old birds and a tibia from 7-week-old birds were cracked and wrapped individually with cheesecloth before defatting with petroleum ether. After drying at 60^{o}C for 12 hours, bones were removed from cheesecloth, placed into crucibles and then ashed in a muffle furnace at 600^{o}C for 12 hours. Bone ash was ground and about 1 gram solubilized in 3 ml of 6 N hydrochloric acid before further diluting with deionized water. Analysis of calcium, zinc, and manganese were done using a Unicam Solaar 969 atomic absorption spectrometer, while colorimetrical analysis of phosphorus was done using a BioMate 5 spectrometer.

Calcium and Phosphorus Retention Determination

The collected excreta was defrosted at room temperature, pooled, dried at 50^{o}C, weighed, and ground. Excreta samples and grower diet samples were weighed and about 1 gram/sample digested with hydrochloric acid before further diluting with deionized

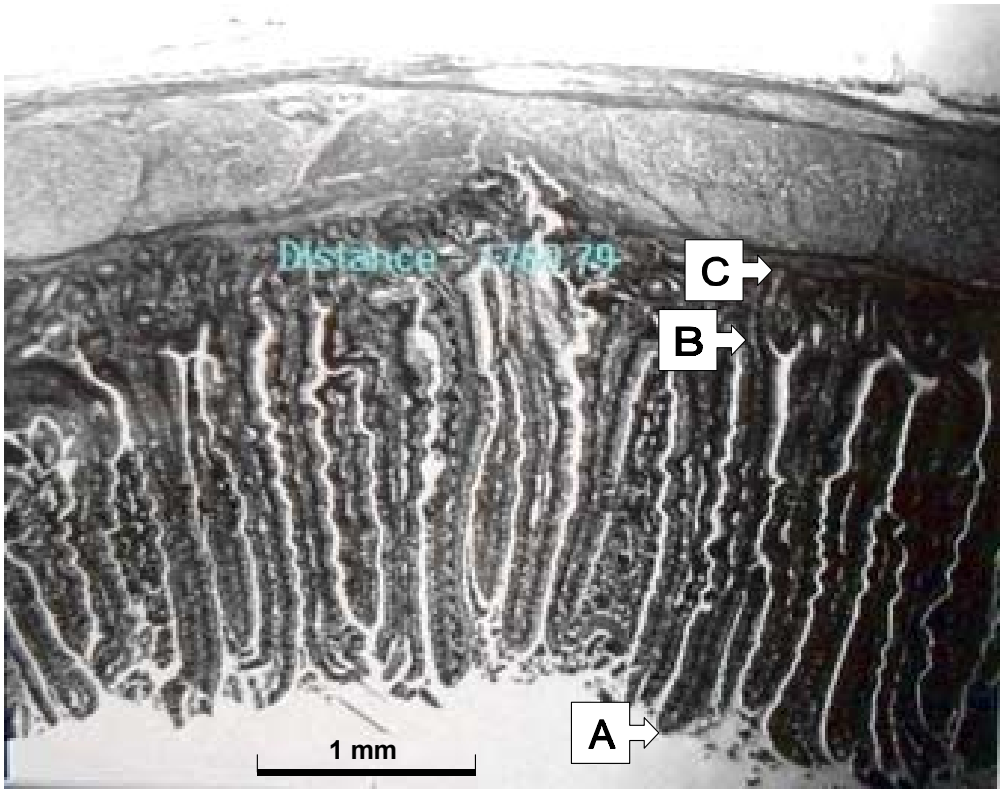


Figure 1. Villi height and crypt depth[1] measurement using MetaMorph Imaging System.

[1] Villi height measured from the distance along the line between villus tip (point A) and the bottom of the villus (point B), and crypt depth measured from the distance along the line between bottom of the villus (point B) to the deepest part of the crypt (point C), respectively

water. Analysis of calcium was done using a Unicam Solaar 969 atomic absorption spectrometer, while colorimetrical analysis of total phosphorus was done using a BioMate 5 spectrometer. Calcium retention was calculated during 5-day excreta collection as follows:

$$\frac{\text{Total calcium in feed} - \text{Total calcium in excreta}}{\text{Total calcium in feed}} \times 100$$

Phosphorus retention was calculated similarly.

Apparent Metabolizable Energy Determination

After drying at 50^{o}C and grinding, gross energy of excreta samples as well as grower diet samples were determined using an oxygen bomb calorimeter. Apparent metabolizable energy was calculated during 5-day excreta collection as total gross energy of feed minus total gross energy of excreta.

Feed Cost per Weight Gain Determination

Price of feed ingredients used in each feed formulation was used for calculation of feed cost. Feed costs per weight gain production of birds in each replicate during 0-3 week of age were calculated as feed costs of starter diets multiplied by feed conversion ratio of birds in each replicate during 0-3 week of age. Feed cost per weight gain of each bird during 3-7 week of age was calculated as follows:

$$\frac{[(\text{Feed intake 3-6 wk}) \times (\text{Grower diet cost})] + [(\text{Feed intake 6-7 wk}) \times (\text{Finisher diet cost})]}{(\text{Weight gain 3-7 wk})}$$

Finally, feed cost per weight gain of each bird during 0-7 week of age was calculated by adding [(average of feed intake 0-3 week of each treatment) **x** (cost of starter diet)] to the equation above, then dividing by [(average weight gain 0-3 week of each treatment) + (weight gain 3-7 week)].

Experimental Design and Statistical Analysis

Analysis of variance was performed on all data using the mixed model analysis of SAS software (mixed procedure of SAS, 2001). For the first 21 days, the pens were used as replicates. For the heat stress portion of the study, each bird was used as a replicate. Least square treatment means were compared and differences were considered significant at the 5% level of probability.

Results

Growth Performance

Average body weight, weight gain, feed intake, and feed conversion ratio of 3-week-old broilers are presented in Table 6. Feed intake of birds was not affected by the experimental diets, while the level of defatted rice bran inclusion significantly influenced body weight and weight gain of broilers ($P<0.01$). Chicks fed 25% defatted rice bran diets without phytase supplementation had the highest weight and significantly higher

Table 6. Body weight, weight gain, feed intake, and feed conversion ratio of 3-week-old broilers fed different experimental diets.[1]

Treatment[2]	Body weight (g)	Weight gain (g)	Feed intake (g)	Feed conversion Ratio
1	587.36[d]	547.27[d]	1237.92	2.27[a]
2	719.05[c]	680.12[c]	1260.90	1.86[b]
3	731.87[c]	691.94[c]	1278.65	1.85[b]
4	831.59[a]	791.74[a]	1234.91	1.56[c]
5	785.01[b]	745.24[b]	1196.34	1.60[c]
Pooled SEM	5.67	5.64	11.98	0.02
Probabilities	<0.0001	<0.0001	0.3547	<0.0001

[1] Values are least square treatment means
[2] Treatment 1 = basal diet: 25% corn starch
Treatment 2 = diet with 15% corn starch, 10% defatted rice bran
Treatment 3 = diet with 15% corn starch, 10% defatted rice bran + phytase
Treatment 4 = diet with 25% defatted rice bran
Treatment 5 = diet with 25% defatted rice bran + phytase
[a, b, c, d] Means within columns with different superscripts differ significantly (P<0.05)

than chicks fed 25% defatted rice bran diet supplemented with phytase. Chicks fed 10% defatted rice bran inclusion had lower weight but higher than chicks fed the control diet. Feed conversion ratio was also significantly improved with increasing defatted rice bran level ($P<0.01$). Phytase supplementation did not improve growth performance of the chicks.

The growth performance of birds for the period in the temperature-controlled chambers is shown in Table 7. The total weight gain, feed intake, and feed conversion ratio of broilers during 0-7 week of age are shown in Table 8 and Figure 2. Significant improvements ($P<0.01$) in body weight, weight gain, and feed conversion ratio were observed in 7-week-old birds when defatted rice bran was included in diets. Again, addition of phytase did not impact growth performance of the birds. Heat stress lowered weight gain and feed intake of broilers during 3-7 week of age by approximately 20.60 and 20.09%, respectively. However, feed conversion ratio was not affected by temperature treatments.

Water Intake and Digesta Retention Time

Increased levels of defatted rice bran in the diets caused a significant increase ($P<0.01$) in daily water intake and high level of defatted rice bran significantly increased ($P=0.01$) water:feed consumption ratio (Table 9). Phytase addition did not improve water intake. Although it tended to decrease water:feed consumption ratio, the differences were not significant (Figure 3). Birds under heat stress environment consumed approximately 20.6% more water than birds under thermoneutral environment ($P<0.01$). Water:feed

Table 7. Body weight at 7 week of age, weight gain, feed intake, and feed conversion ratio during 3-7 week of age of broilers fed different experimental diets under thermoneutral and heat stress conditions.[1]

Treatment	Temp.[2]	Body weight (g)	Weight gain (g)	Feed intake (g)	Feed conversion ratio
1	TN	2037.93	1354.32	2656.98	1.94
	HS	1790.58	1116.33	2188.24	1.97
2	TN	2328.44	1561.00	2972.18	1.92
	HS	2068.51	1274.02	2419.17	1.91
3	TN	2406.78	1574.42	3145.47	1.98
	HS	2062.36	1254.38	2444.54	1.89
4	TN	2842.47	1977.25	3464.58	1.76
	HS	2405.20	1542.82	2711.95	1.75
5	TN	2821.02	1984.87	3501.44	1.77
	HS	2379.72	1523.17	2814.22	1.79
Pooled SEM		15.18	12.55	24.39	0.01
Source of Variation		----------Probabilities----------			
Diet		<0.0001	<0.0001	0.0004	0.0002
Temp.		<0.0001	<0.0001	<0.0001	0.5998
Diet x Temp		0.2562	0.1259	0.6415	0.4883
Main Effect Means					
Diet					
1		1914.25[c]	1235.33[c]	2422.61[c]	1.96[a]
2		2198.47[b]	1417.51[b]	2695.68[b]	1.92[a]
3		2234.57[b]	1414.40[b]	2795.00[b]	1.94[a]
4		2623.83[a]	1760.03[a]	3088.27[a]	1.75[b]
5		2600.37[a]	1754.02[a]	3157.83[a]	1.78[b]
Temp.					
TN		2487.33[a]	1690.37[a]	3148.13[a]	1.87
HS		2141.27[b]	1342.15[b]	2515.62[b]	1.86

[1] Values are least square treatment means
[2] TN = thermoneutral environment (23.9°C), HS = heat stress environment (23.9-35°C)
[a, b, c] Main effect means within columns with different superscripts differ significantly (P<0.05)

Table 8. Total weight gain, feed intake, and feed conversion ratio during 0-7 week of age of broilers fed different experimental diets under thermoneutral and heat stress conditions.[1]

Treatment	Temp.[2]	Weight gain (g)	Feed intake (g)	Feed conversion ratio
Main Effect Means				
Diet				
1		1874.02[c]	3660.69[c]	1.96[a]
2		2146.49[b]	3974.14[b]	1.84[b]
3		2184.50[b]	4100.84[ab]	1.87[b]
4		2551.38[a]	4295.04[a]	1.69[c]
5		2558.00[a]	4301.05[a]	1.71[c]
Temp.				
TN		2446.81[a]	4408.79[a]	1.83
HS		2078.95[b]	3723.91[b]	1.80
Source of Variation		----------Probabilities----------		
Diet		<0.0001	0.0003	<0.0001
Temp.		<0.0001	<0.0001	0.2482
Diet x Temp		0.1925	0.3698	0.4311
Pooled SEM		15.73	25.91	0.01

[1] Values are least square treatment means
[2] TN = thermoneutral environment (23.9°C), HS = heat stress environment (23.9-35°C)
[a, b, c] Main effect means within columns with different superscripts differ significantly (P<0.05)

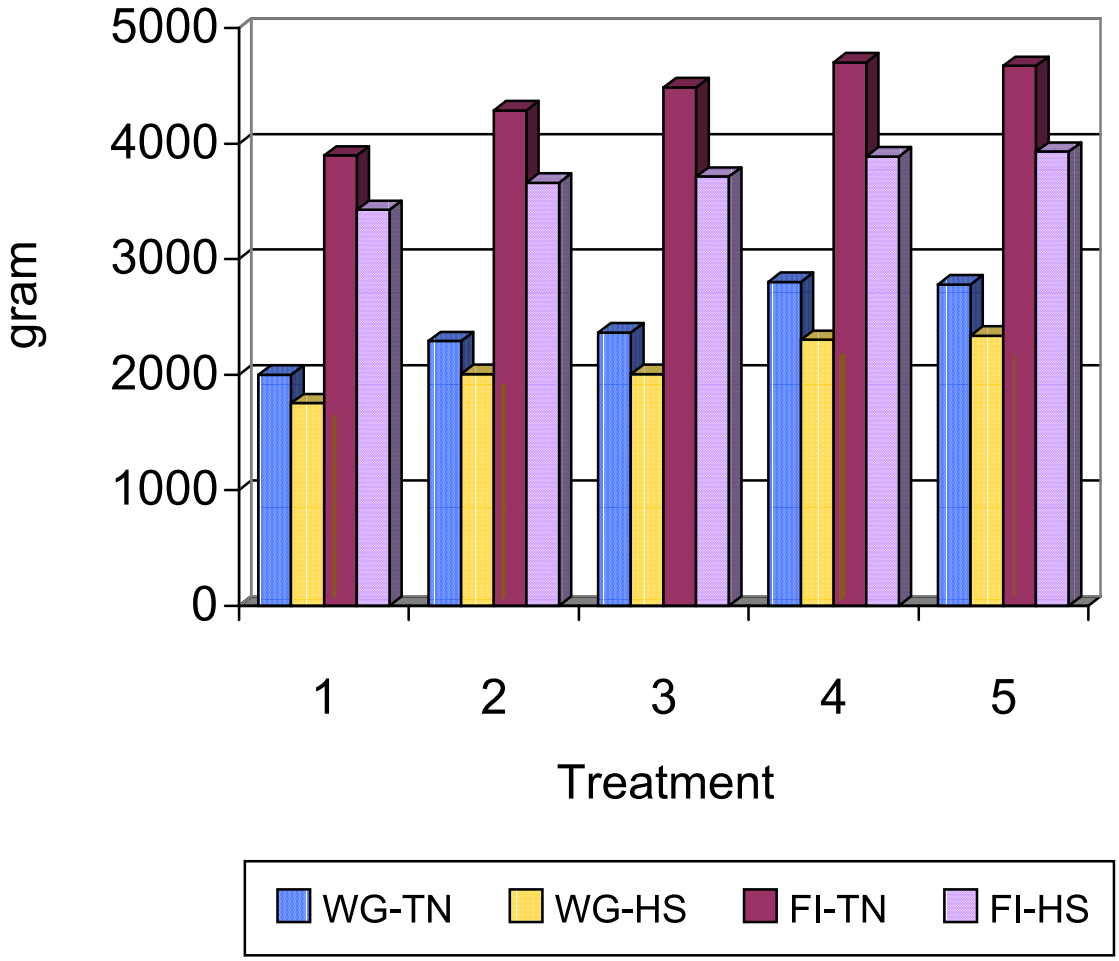


Figure 2. Weight gain (WG) and feed intake (FI) of 7-week-old broilers[1] fed different experimental diets[2] under thermoneutral (TN) and heat stress (HS) conditions.[3]

[1] Values are least square treatment means

[2] Treatment 1 = basal diet: 25% corn starch
Treatment 2 = diet with 15% corn starch, 10% defatted rice bran
Treatment 3 = diet with 15% corn starch, 10% defatted rice bran + phytase
Treatment 4 = diet with 25% defatted rice bran
Treatment 5 = diet with 25% defatted rice bran + phytase

[3] TN = thermoneutral environment (23.9°C), HS = heat stress environment (23.9-35°C)

Table 9. Main effect means of daily water intake and water:feed consumption ratio of broilers during 3-7 week of age, and digesta retention time of 5-week-old broilers fed different experimental diets under thermoneutral and heat stress conditions.[1]

	Daily water intake (ml)	Water:feed consumption ratio	Digesta retention time (minute)
Main Effect Means			
Diet			
1	157.42[c]	1.80[c]	238.26
2	190.89[b]	1.96[bc]	223.21
3	197.32[b]	1.95[bc]	233.92
4	226.53[a]	2.18[a]	211.18
5	232.52[a]	2.15[ab]	219.32
Temp.[2]			
TN	182.14[b]	1.58[b]	221.97
HS	219.73[a]	2.43[a]	228.38
Source of Variation	----------Probabilities----------		
Diet	0.0010	0.0123	0.0761
Temp.	0.0008	<0.0001	0.2825
Diet x Temp	0.6837	0.8949	0.6047
Pooled SEM	3.22	0.03	2.77

[1] Values are least square treatment means
[2] TN = thermoneutral environment (23.9°C), HS = heat stress environment (23.9-35°C)
[a, b, c] Main effect means within columns with different superscripts differ significantly (P<0.05)

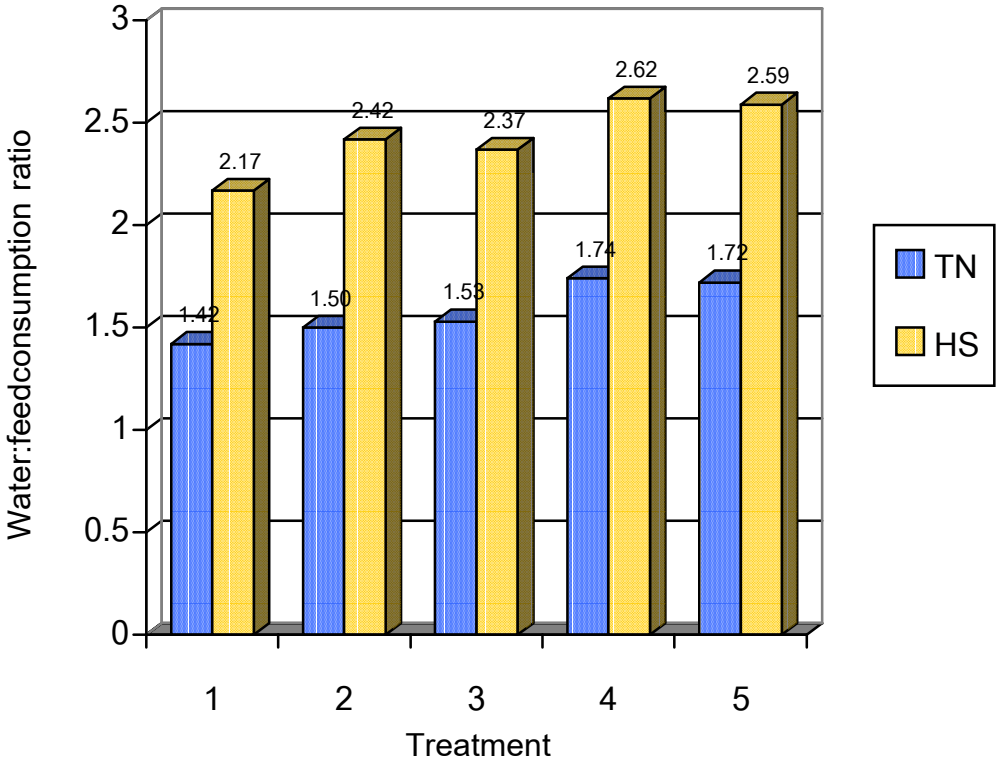


Figure 3. Water:feed consumption ratio of broilers[1] during 3-7 week of age fed different experimental diets[2] under thermoneutral (TN) and heat stress (HS) conditions.[3]

[1] Values are least square treatment means

[2] Treatment 1 = basal diet: 25% corn starch
Treatment 2 = diet with 15% corn starch, 10% defatted rice bran
Treatment 3 = diet with 15% corn starch, 10% defatted rice bran + phytase
Treatment 4 = diet with 25% defatted rice bran
Treatment 5 = diet with 25% defatted rice bran + phytase

[3] TN = thermoneutral environment (23.9^{o}C), HS = heat stress environment (23.9-35^{o}C)

consumption ratio was also increased significantly by 53.8% under heat stress condition ($P<0.01$).

Digesta retention time of 5-week-old broilers tended to decrease when more defatted rice bran was added in the diets, however, the differences were not significant (Table 9, Figure 4). No differences were found when phytase was added to the diets. Birds under heat stress condition tended to have longer digesta retention time, but the difference was not significant either.

Carcass Quality

Carcass percentage of 7-week-old birds was not affected by dietary or temperature treatments (Table 10), while abdominal fat was significantly increased in broilers fed diets containing defatted rice bran ($P<0.01$). Inclusion of phytase did not affect percent abdominal fat, where heat stress tended ($P=0.11$) to decrease percent abdominal fat content (2.47 vs 2.79%).

Differences in market portions of bird are shown in Table 11. The high level of defatted rice bran caused a significant increase ($P<0.01$) in percentage of thighs, while no differences were found between control diet and 10% defatted rice bran diets. Other market portions of birds were not impacted by levels of defatted rice bran inclusion. Phytase did not improve percentage of any carcass portions. Heat stress was found to significantly decrease percentage of breast by 4.1% ($P=0.01$), while significantly increasing percentage of legs 5.5% ($P<0.01$), thighs 3.8% ($P=0.02$), and wings 3.3% ($P=0.05$), respectively.

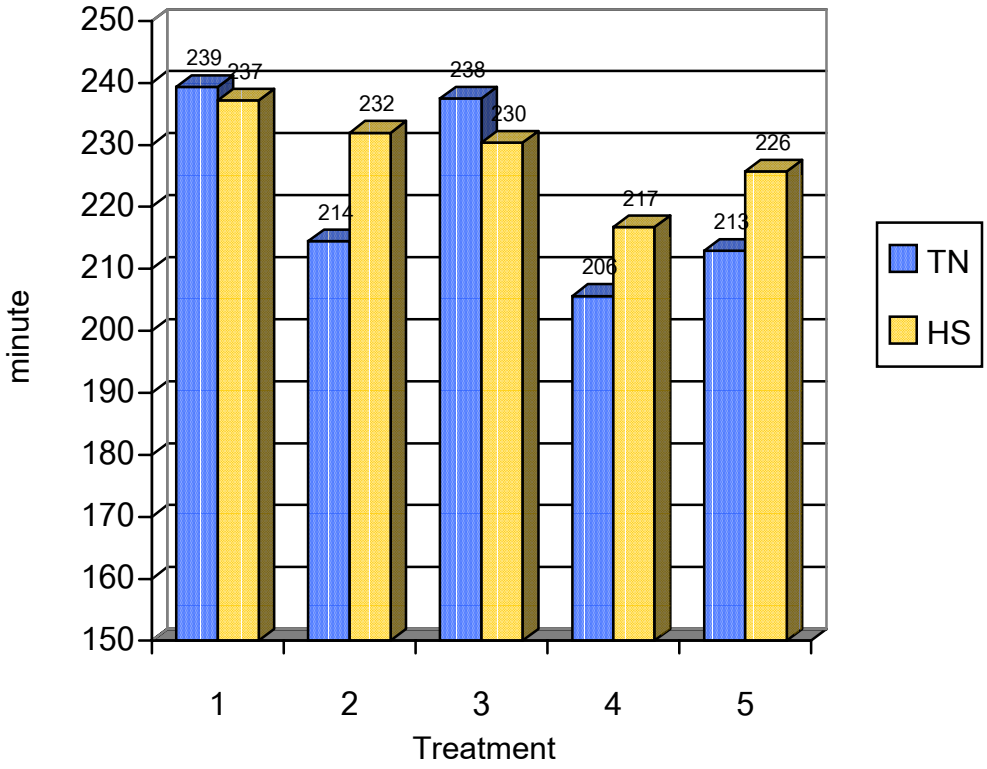


Figure 4. Digesta retention time of 5-week-old broilers[1] fed different experimental diets[2] under thermoneutral (TN) and heat stress (HS) conditions.[3]

[1] Values are least square treatment means

[2] Treatment 1 = basal diet: 25% corn starch
Treatment 2 = diet with 15% corn starch, 10% defatted rice bran
Treatment 3 = diet with 15% corn starch, 10% defatted rice bran + phytase
Treatment 4 = diet with 25% defatted rice bran
Treatment 5 = diet with 25% defatted rice bran + phytase

[3] TN = thermoneutral environment (23.9^{o}C), HS = heat stress environment (23.9-35^{o}C)

Table 10. Carcass, abdominal fat, and intestinal weight as percentages of live weight of 7-week-old broilers fed different experimental diets under thermoneutral and heat stress conditions.[1]

Treatment	Temp.[2]	Carcass (%)	Abdominal fat (%)	Intestine (%)
1	TN	69.16	2.01	1.38
	HS	68.71	2.00	1.33
2	TN	67.90	2.77	1.33
	HS	69.77	2.16	1.29
3	TN	68.73	2.32	1.37
	HS	68.50	2.45	1.18
4	TN	69.61	3.24	1.28
	HS	68.43	2.75	1.24
5	TN	68.58	3.59	1.35
	HS	67.58	3.00	1.24
Pooled SEM		0.15	0.06	0.02
Source of Variation		----------Probabilities----------		
Diet		0.3917	0.0086	0.6832
Temp.		0.5485	0.1056	0.0808
Diet x Temp		0.0776	0.5666	0.8021
Main Effect Means				
Diet				
1		68.93	2.00^{c}	1.35
2		68.83	2.47^{bc}	1.31
3		68.61	2.38^{bc}	1.27
4		69.02	3.00^{ab}	1.26
5		68.08	3.30^{a}	1.30
Temp.				
TN		68.79	2.79	1.34
HS		68.60	2.47	1.26

[1] Values are least square treatment means
[2] TN = thermoneutral environment (23.9°C), HS = heat stress environment (23.9-35°C)
[a, b, c] Main effect means within columns with different superscripts differ significantly ($P<0.05$)

Table 11. Breast, legs, thighs, and wings weight as percentages of carcass weight of 7-week-old broilers fed different experimental diets under thermoneutral and heat stress conditions.[1]

Treatment	Temp.[2]	Breast (%)	Legs (%)	Thighs (%)	Wings (%)
1	TN	34.73	14.27	16.03	12.48
	HS	34.93	14.60	16.13	12.76
2	TN	36.40	13.69	15.99	12.15
	HS	34.31	14.71	17.04	12.28
3	TN	35.96	13.50	15.83	12.10
	HS	34.59	13.98	16.54	12.68
4	TN	34.97	13.02	17.05	11.33
	HS	32.91	14.35	17.86	12.38
5	TN	35.81	13.21	17.41	12.09
	HS	33.82	13.78	17.89	12.07
Pooled SEM		0.19	0.09	0.11	0.07
Source of Variation		----------Probabilities----------			
Diet		0.3954	0.0863	0.0045	0.1549
Temp.		0.0127	0.0055	0.0222	0.0481
Diet x Temp		0.5211	0.5504	0.7295	0.4032
Main Effect Means					
Diet					
1		34.83	14.43	16.08[b]	12.62
2		35.36	14.20	16.52[b]	12.21
3		35.27	13.74	16.18[b]	12.39
4		33.94	13.68	17.46[a]	11.85
5		34.81	13.49	17.65[a]	12.08
Temp.					
TN		35.57[a]	13.54[b]	16.46[b]	12.03[b]
HS		34.11[b]	14.28[a]	17.09[a]	12.43[a]

[1] Values are least square treatment means
[2] TN = thermoneutral environment (23.9°C), HS = heat stress environment (23.9-35°C)
[a, b] Main effect means within columns with different superscripts differ significantly ($P<0.05$)

Gross Anatomical Observations of Intestine

Intestinal weight as percentage of live weight of broilers was not influenced by treatment (Table 10). The weight percentage of different parts of intestine, as shown in Table 12, was not different between dietary treatments. Ceca weight percentage was influenced by environmental temperature (P=0.03), with heat stress lowering ceca weight by approximately 9.9%. The interaction between dietary treatments and temperature regimen was found in colon weight percentage (P=0.04). Weight percentage of birds raised under thermoneutral condition was increased when defatted rice bran was included in diets. Phytase supplementation was found to lower colon weight to the same level as control group. In contrast, defatted rice bran diets caused a decrease in weight percentage of colon in heat stressed broilers. Adding phytase improved weight percentage of colon of those birds.

Intestinal Villi Height and Crypt Depth

No differences between treatments were found in villi height, crypt depth, and villi height:crypt depth ratio of both duodenum (Table 13) and jejunum (Table 14).

Tibia Mineral Content

Tibia dry matter of both 3-week-old and 7-week-old broilers was not affected by dietary treatment (Table 15 and 17, respectively). Percent tibia ash and mineral content of 3-week-old broilers were significantly affected by dietary treatments (Table 15 and 16). Percent ash was significantly decreased in birds fed 25% defatted rice bran diets

Table 12. Duodenum, jejunum, ileum, ceca, and colon weight as percentages of intestinal weight of 7-week-old broilers fed different experimental diets under thermoneutral and heat stress conditions.[1]

Treatment	Temp.[2]	Duodenum (%)	Jejunum (%)	Ileum (%)	Ceca (%)	Colon (%)
1	TN	17.26	33.24	27.48	14.76	7.26^{c}
	HS	17.51	32.66	25.40	14.02	8.80^{abc}
2	TN	16.49	31.72	27.17	15.28	9.35^{ab}
	HS	18.93	35.31	25.70	12.58	7.48^{bc}
3	TN	17.49	32.04	27.31	14.21	8.96^{abc}
	HS	16.68	32.90	27.08	14.38	8.96^{abc}
4	TN	17.49	30.13	27.93	14.97	9.48^{a}
	HS	20.52	33.20	27.61	11.13	7.54^{bc}
5	TN	19.00	31.44	27.71	13.30	7.43^{abc}
	HS	19.72	31.95	26.33	13.22	8.71^{abc}
Pooled SEM		0.17	0.28	0.23	0.19	0.17
Source of Variation		----------Probabilities----------				
Diet		0.1196	0.4072	0.4430	0.4596	0.5553
Temp.		0.0820	0.0561	0.0544	0.0264	0.6179
Diet x Temp		0.2768	0.3189	0.7238	0.1618	0.0427
Main Effect Means						
Diet						
1		17.38	32.95	26.44	14.39	8.03
2		17.71	33.51	26.43	13.93	8.41
3		17.08	32.47	27.19	14.30	8.96
4		19.01	31.66	27.77	13.05	8.51
5		19.36	31.70	27.02	13.26	8.07
Temp.						
TN		17.54	31.71	27.52	14.51^{a}	8.49
HS		18.67	33.20	26.42	13.07^{b}	8.30

[1] Values are least square treatment means
[2] TN = thermoneutral environment (23.9^{o}C), HS = heat stress environment (23.9-35^{o}C)
[a, b, c] Main effect means within columns with different superscripts differ significantly ($P<0.05$)

Table 13. Duodenal villi height, crypt depth, and villi height:crypt depth ratio of 7-week-old broilers fed different experimental diets under thermoneutral and heat stress conditions.[1]

Treatment	Temp.[2]	Villi height (mm)	Crypt depth (mm)	Villi height: crypt depth ratio
1	TN	2.42	0.36	7.09
	HS	2.33	0.31	8.10
2	TN	2.79	0.37	7.78
	HS	2.68	0.34	8.00
3	TN	2.41	0.34	7.34
	HS	2.63	0.40	6.78
4	TN	2.64	0.40	7.00
	HS	3.02	0.40	7.70
5	TN	2.74	0.34	8.31
	HS	2.94	0.38	7.42
Pooled SEM		0.04	0.006	0.15
Source of Variation		----------Probabilities----------		
Diet		0.2116	0.4996	0.7638
Temp.		0.3993	0.7864	0.8401
Diet x Temp		0.7390	0.5459	0.6516
Main Effect Means				
Diet				
1		2.38	0.34	7.59
2		2.73	0.36	7.89
3		2.52	0.37	7.06
4		2.83	0.40	7.34
5		2.84	0.36	7.86
Temp.				
TN		2.60	0.36	7.50
HS		2.72	0.37	7.60

[1] Values are least square treatment means
[2] TN = thermoneutral environment (23.9°C), HS = heat stress environment (23.9-35°C)

Table 14. Jejunal villi height, crypt depth, and villi height:crypt depth ratio of 7-week-old broilers fed different experimental diets under thermoneutral and heat stress conditions.[1]

Treatment	Temp.[2]	Villi height (mm)	Crypt depth (mm)	Villi height: crypt depth ratio
1	TN	2.28	0.28	8.29
	HS	2.42	0.31	7.89
2	TN	2.26	0.33	6.88
	HS	2.45	0.33	7.64
3	TN	2.47	0.35	7.55
	HS	2.29	0.34	6.92
4	TN	2.06	0.33	6.42
	HS	2.24	0.35	6.49
5	TN	2.26	0.30	7.57
	HS	2.25	0.31	7.35
Pooled SEM		0.04	0.005	0.15
Source of Variation		----------Probabilities----------		
Diet		0.7575	0.1231	0.1912
Temp.		0.6248	0.4640	0.8262
Diet x Temp		0.8477	0.8068	0.8031
Main Effect Means				
Diet				
1		2.35	0.30	8.09
2		2.35	0.33	7.26
3		2.38	0.34	7.23
4		2.15	0.34	6.45
5		2.25	0.31	7.46
Temp.				
TN		2.27	0.32	7.34
HS		2.33	0.33	7.26

[1] Values are least square treatment means
[2] TN = thermoneutral environment (23.9°C), HS = heat stress environment (23.9-35°C)

Table 15. Dry matter, ash, calcium and phosphorus content, and calcium:phosphorus ratio in tibia of 3-week-old broilers fed different experimental diets.[1]

Treatment	Dry matter (%)	Ash (%)	Calcium (%)	Phosphorus (%)	Calcium:phosphorus ratio
1	87.58	60.61[a]	37.01[a]	21.42[a]	1.71[a]
2	87.37	60.08[ab]	35.88[b]	20.33[c]	1.73[a]
3	87.35	59.99[ab]	34.03[c]	20.95[b]	1.62[b]
4	87.31	59.41[bc]	34.75[c]	21.16[ab]	1.63[b]
5	87.75	58.80[c]	34.91[bc]	21.56[a]	1.60[b]
Pooled SEM	0.06	0.13	0.17	0.07	0.008
Probabilities	0.0690	0.0006	<0.0001	<0.0001	<0.0001

[1] Values are least square treatment means
[a, b, c] Means within columns with different superscripts differ significantly (P<0.05)

Table 16. Zinc and manganese content in tibia of 3-week-old broilers fed different experimental diets.[1]

Treatment	Zinc (ug/g)	Manganese (ug/g)
1	442.91[b]	10.16[ab]
2	443.26[b]	9.46[c]
3	473.35[a]	10.40[a]
4	448.10[b]	9.75[bc]
5	436.53[b]	9.66[bc]
Pooled SEM	3.28	0.10
Probabilities	0.0084	0.0288

[1] Values are least square treatment means
[a, b, c] Means within columns with different superscripts differ significantly (P<0.05)

Table 17. Dry matter, ash, calcium and phosphorus content, and calcium:phosphorus ratio in tibia of 7-week-old broilers fed different experimental diets under thermoneutral and heat stress conditions.[1]

Treatment	Temp.[2]	Dry matter (%)	Ash (%)	Calcium (%)	Phosphorus (%)	Calcium:phosphorus ratio
1	TN	85.73	59.48	35.97	18.29	1.97
	HS	85.95	58.79	35.76	18.53	1.93
2	TN	84.31	58.23	34.97	17.68	1.98
	HS	83.94	58.27	36.01	18.78	1.92
3	TN	87.77	57.99	35.73	16.19	2.17
	HS	86.06	58.41	35.97	16.89	2.13
4	TN	84.26	58.12	36.42	16.05	2.27
	HS	82.51	58.10	36.20	17.24	2.14
5	TN	86.85	57.61	36.59	18.20	1.98
	HS	85.70	59.46	35.93	19.08	1.91
Pooled SEM		0.45	0.15	0.10	0.05	0.006
Source of Variation		----------Probabilities----------				
Diet		0.2213	0.3190	0.2741	0.0017	<0.0001
Temp.		0.3692	0.3448	0.8704	0.0151	0.0118
Diet x Temp		0.9583	0.2203	0.3173	0.8226	0.6718
Main Effect Means						
Diet						
1		85.84	59.13	35.86	18.41[a]	1.95[b]
2		84.12	58.25	35.49	18.23[a]	1.95[b]
3		86.92	58.20	35.85	16.54[b]	2.15[a]
4		83.39	58.11	36.31	16.64[b]	2.20[a]
5		86.27	58.53	36.26	18.64[a]	1.94[b]
Temp.						
TN		85.78	58.29	35.93	17.28[b]	2.07[a]
HS		84.83	58.60	35.98	18.10[a]	2.01[b]

[1] Values are least square treatment means
[2] TN = thermoneutral environment (23.9°C), HS = heat stress environment (23.9-35°C)
[a, b] Main effect means within columns with different superscripts differ significantly (P<0.05)

($P<0.01$). Phytase supplementation did not improve percent tibia ash of birds on defatted rice bran diets.

Tibia calcium was significantly higher in control group ($P<0.01$). The inclusion of defatted rice bran in diets decreased percent calcium. Phytase supplementation lowered calcium in chicks fed 10% defatted rice bran diet by 5.2%, but did not affect chicks fed 25% defatted rice bran diet. Tibia phosphorus was significantly lower in chicks fed 10% defatted rice bran diet ($P<0.01$). Including phytase in that diet caused a significant increase in phosphorus by 3%, but the phosphorus content was still lower than in the control group. At 25% defatted rice bran diets, no differences were found compared to control groups. There was no significant difference resulting from phytase supplementation of this diet. From the calculation of calcium:phosphorus ratio in tibia, chicks fed the control diet and chicks fed 10% defatted rice bran diet without phytase supplementation had significantly higher ratio than chicks fed other diets ($P<0.01$).

Significant difference in tibia zinc content was only found in chicks fed 10% defatted rice bran diet supplemented with phytase. There was approximately a 6.9% ($P<0.01$) improvement compared to the other dietary treatments. Defatted rice bran inclusion lowered ($P=0.03$) the amount of manganese in tibia of birds fed the 10% defatted rice bran level. Phytase supplementation, again, significantly improved manganese level in 10% defatted rice bran diet by 9.9%, but did not affect manganese content of the 25% defatted rice bran diet.

No significant differences between treatments were found in percent tibia ash, percent calcium, and zinc content of 7-week-old broilers, while both dietary and temperature treatments significantly affected phosphorus and manganese content of the tibia (Table 17 and 18). Percent phosphorus was significantly lowered ($P<0.01$) in the tibia of birds fed 10% defatted rice bran diet supplemented with phytase and 25% defatted rice bran diet without phytase compared to the other dietary treatments. Those results led to significantly higher calcium:phosphorus ratio in tibia ($P<0.01$). Birds raised under heat stress condition had significantly higher percentage of phosphorus approximately 4.7% ($P=0.02$). As a result, calcium:phosphorus ratio of birds raised under heat stress was significantly lower ($P=0.01$). Manganese content in tibia was affected by the high level of defatted rice bran in diets ($P<0.01$). Phytase inclusion in the 25% defatted rice bran diet however did not affect the manganese content. Heat stress lowered manganese content in tibia by 4.9% ($P=0.04$).

Calcium and Phosphorus Retention

Amount of excreta (dry matter basis) as percentage of feed consumed by 5-week-old broilers is shown in Figure 5. Birds fed 25% defatted rice bran diets had higher amount of excrata ($P<0.01$). The results from analysis of calcium and phosphorus in excreta of 5-week-old broilers are presented in Table 19 and Figure 6. The significant differences between dietary treatments were observed in amount of excreta, percent calcium, percent phosphorus, and their retentions. No differences were found between temperature treatments in all criteria examined.

Table 18. Zinc and manganese content in tibia of 7-week-old broilers fed different experimental diets under thermoneutral and heat stress conditions.[1]

Treatment	Temp.[2]	Zinc (ug/g)	Manganese (ug/g)
1	TN	334.02	12.44
	HS	343.34	11.87
2	TN	351.75	12.47
	HS	358.52	11.67
3	TN	350.05	12.59
	HS	327.68	12.38
4	TN	331.01	11.38
	HS	342.57	10.17
5	TN	330.75	11.24
	HS	328.72	11.06
Pooled SEM		2.24	0.12
Source of Variation		----------Probabilities----------	
Diet		0.0722	0.0089
Temp.		0.8938	0.0422
Diet x Temp		0.2281	0.6829
Main Effect Means			
Diet			
1		338.68	12.16[a]
2		355.13	12.07[a]
3		338.86	12.48[a]
4		336.79	10.78[b]
5		329.73	11.15[b]
Temp.			
TN		339.52	12.02[a]
HS		340.16	11.43[b]

[1] Values are least square treatment means
[2] TN = thermoneutral environment (23.9°C), HS = heat stress environment (23.9-35°C)
[a, b] Main effect means within columns with different superscripts differ significantly (P<0.05)

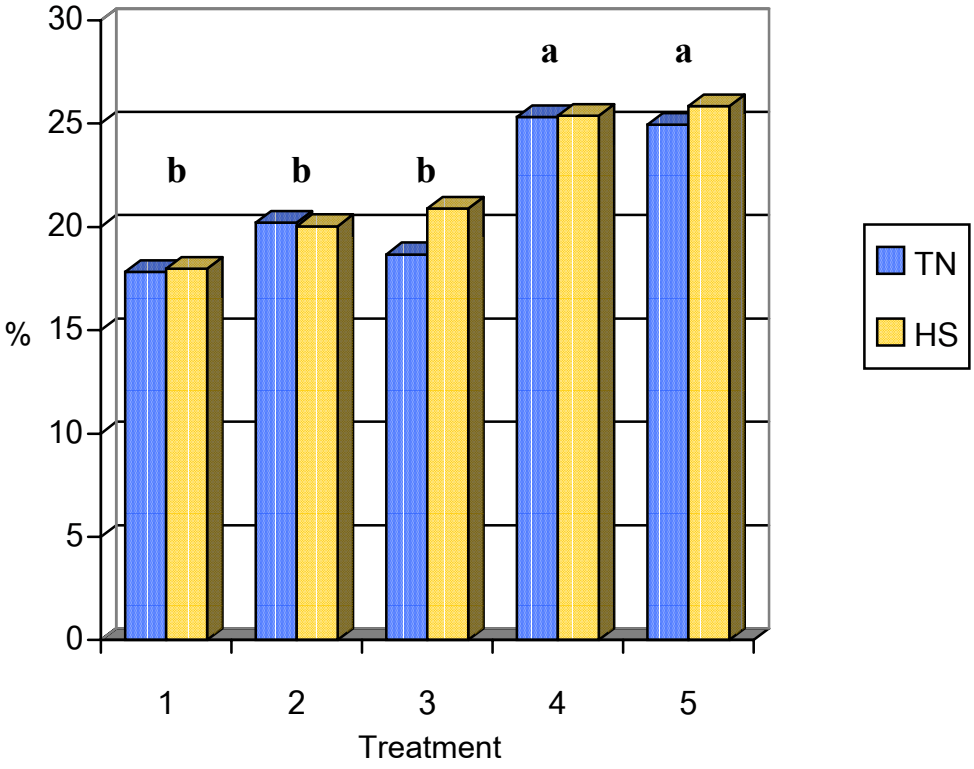


Figure 5. Amount of excreta (dry matter basis) as percentage of feed consumed by 5-week-old broilers[1] fed different experimental diets[2] under thermoneutral (TN) and heat stress (HS) conditions.[3]

[1] Values are least square treatment means, pooled SEM = 0.29

[2] Treatment 1 = basal diet: 25% corn starch
Treatment 2 = diet with 15% corn starch, 10% defatted rice bran
Treatment 3 = diet with 15% corn starch, 10% defatted rice bran + phytase
Treatment 4 = diet with 25% defatted rice bran
Treatment 5 = diet with 25% defatted rice bran + phytase

[3] TN = thermoneutral environment (23.9°C), HS = heat stress environment (23.9-35°C)

[a, b] Main effect means of dietary treatments with different superscripts differ significantly (P=0.0028)

Table 19. Percent calcium and phosphorus in excreta, and their retention by 5-week-old broilers fed different experimental diets under thermoneutral and heat stress conditions.[1]

Treatment	Temp.[2]	Calcium (%)	Calcium retention (%)	Phosphorus (%)	Phosphorus retention (%)
Main Effect Means					
Diet					
1		3.84[a]	37.53[b]	2.09[c]	43.07[a]
2		2.86[b]	51.36[a]	2.34[c]	46.18[a]
3		2.92[b]	49.91[a]	2.15[c]	47.25[a]
4		2.34[c]	29.49[b]	2.96[a]	31.91[b]
5		2.29[c]	34.53[b]	2.66[b]	33.15[b]
Temp.					
TN		2.84	42.77	2.44	41.97
HS		2.86	38.36	2.44	38.66
Source of Variation		----------Probabilities----------			
Diet		<0.0001	0.0012	0.0001	0.0104
Temp.		0.7290	0.1106	0.9710	0.2328
Diet x Temp		0.7476	0.3291	0.9396	0.9444
Pooled SEM		0.02	1.02	0.03	1.13

[1] Values are least square treatment means
[2] TN = thermoneutral environment (23.9°C), HS = heat stress environment (23.9-35°C)
[a, b, c] Main effect means within columns with different superscripts differ significantly (P<0.05)

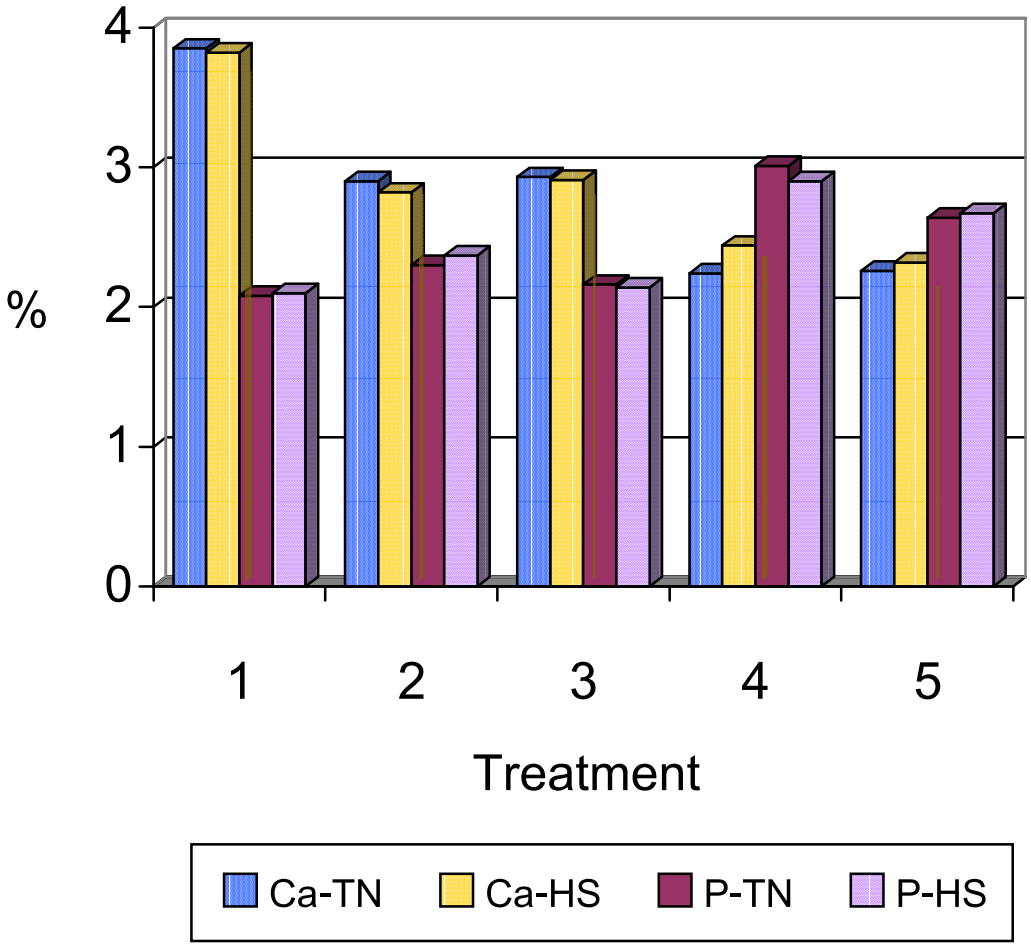


Figure 6. Percent calcium (Ca) and phosphorus (P) in excreta by 5-week-old broilers[1] fed different experimental diets[2] under thermoneutral (TN) and heat stress (HS) conditions.[3]

[1] Values are least square treatment means

[2] Treatment 1 = basal diet: 25% corn starch
Treatment 2 = diet with 15% corn starch, 10% defatted rice bran
Treatment 3 = diet with 15% corn starch, 10% defatted rice bran + phytase
Treatment 4 = diet with 25% defatted rice bran
Treatment 5 = diet with 25% defatted rice bran + phytase

[3] TN = thermoneutral environment (23.9^{o}C), HS = heat stress environment (23.9-35^{o}C)

Amount of excreta was higher in birds fed 25% defatted rice bran diets (P<0.01). The least square means of dietary treatment 1, 2, 3, 4, and 5 were 17.89, 20.12, 19.78, 25.35, and 25.39%, respectively. Excreta calcium was highest in the control diet and lowest in the diets that contained 25% defatted rice bran (P<0.01). Phytase supplementation had no effect on percent calcium. From the calculation of calcium retention, birds fed 10% defatted rice bran diets had higher percent retention (P<0.01). Supplementation of phytase did not change percent calcium retention (Figure 7).

Phosphorus in excreta was highest in broilers fed 25% defatted rice bran diet without phytase supplementation (P<0.01). Phytase addition significantly lowered percent phosphorus in excreta of birds fed 25% defatted rice bran diet by 10.1%, but this amount was still higher than the control group and the 10% defatted rice bran groups. Because of higher phosphorus loss in excreta, phosphorus retention of birds fed 25% defatted rice bran diets was significantly lower than other treatments (P=0.01). Enzyme supplementation had no effect on phosphorus retention.

Apparent Metabolizable Energy

Apparent metabolizable energy of 5-week-old broilers is shown in Table 20 and Figure 8. Dietary treatments caused significantly differences in apparent metabolizable energy (P<0.01). The birds fed 25% defatted rice bran diets had highest apparent metabolizable energy, while birds fed 10% defatted rice bran diets had lowest apparent metabolizable energy. Neither enzyme supplementation nor different temperature treatments affected apparent metabolizable energy.

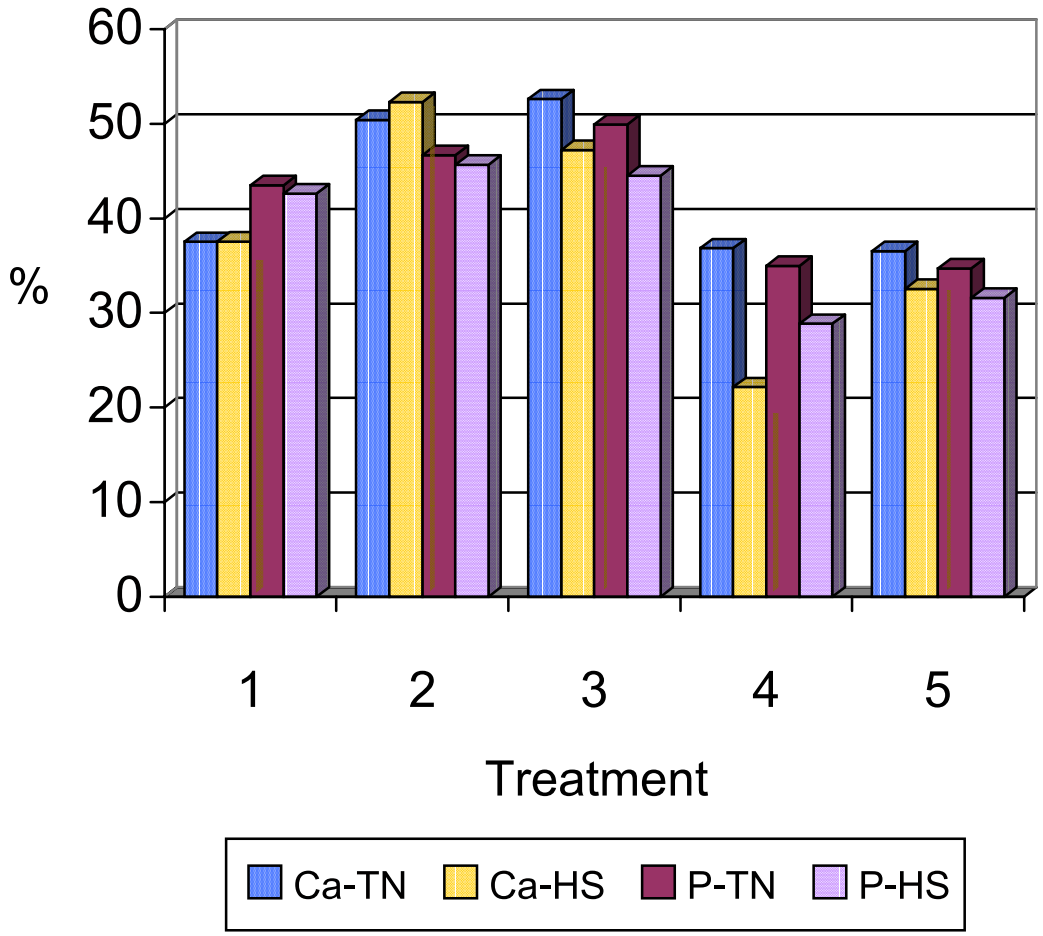


Figure 7. Percent calcium (Ca) and phosphorus (P) retention by 5-week-old broilers[1] fed different experimental diets[2] under thermoneutral (TN) and heat stress (HS) conditions.[3]

[1] Values are least square treatment means

[2] Treatment 1 = basal diet: 25% corn starch
Treatment 2 = diet with 15% corn starch, 10% defatted rice bran
Treatment 3 = diet with 15% corn starch, 10% defatted rice bran + phytase
Treatment 4 = diet with 25% defatted rice bran
Treatment 5 = diet with 25% defatted rice bran + phytase

[3] TN = thermoneutral environment (23.9^{o}C), HS = heat stress environment (23.9-35^{o}C)

Table 20. Apparent metabolizable energy of different experimental diets of 5-week-old broilers under thermoneutral and heat stress conditions.[1]

Treatment	Apparent metabolizable energy (kcal/kg)
Main Effect Means	
Diet	
1	3490.95[bc]
2	3387.05[cd]
3	3316.80[d]
4	3605.23[ab]
5	3651.50[a]
Temp.	
TN	3520.25
HS	3460.36
Source of Variation	----------Probabilities----------
Diet	0.0015
Temp.	0.1469
Diet x Temp	0.8366
Pooled SEM	13.84

[1] Values are least square treatment means
[a, b, c, d] Main effect means with different superscripts differ significantly ($P<0.05$)

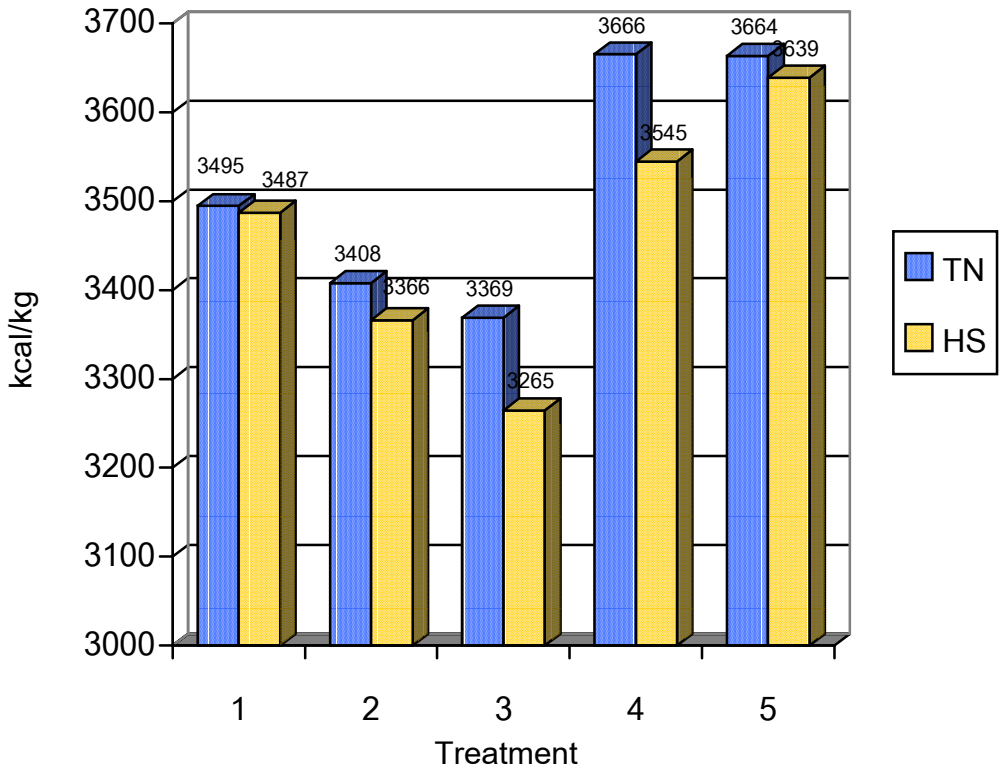


Figure 8. Apparent metabolizable energy[1] of different experimental diets[2] of 5-week-old broilers under thermoneutral (TN) and heat stress (HS) conditions.[3]

[1] Values are least square treatment means

[2] Treatment 1 = basal diet: 25% corn starch
Treatment 2 = diet with 15% corn starch, 10% defatted rice bran
Treatment 3 = diet with 15% corn starch, 10% defatted rice bran + phytase
Treatment 4 = diet with 25% defatted rice bran
Treatment 5 = diet with 25% defatted rice bran + phytase

[3] TN = thermoneutral environment (23.9^{o}C), HS = heat stress environment (23.9-35^{o}C)

Feed Cost per Weight Gain

Feed cost per pound of diet, calculated from the price of feed ingredients used in this experiment, is shown in Table 21. Feed cost per weight gain of birds of different ages is presented in Table 22 and 23. Feed cost per weight gain of birds during 0-3 week of age was decreased in chicks fed defatted rice bran diets ($P<0.01$). These results was similar to the results from birds during 3-7 week of age and 0-7 week of age. Adding phytase to the diets did not affect feed cost per weight gain of birds in any period. Temperature treatments also did not have an impact on feed cost per weight gain of birds.

Discussion

Defatted rice bran contains high amount of phytate. In order to decrease the negative effect of phytate on the growth performance of broilers, increase bioavailability of calcium, phosphorus, zinc, and manganese, and lower phosphorus excretion in birds fed defatted rice bran diets, phytase was added into those diets. Corn starch was used in a control diet and the substitution of corn starch with 10 and 25% defatted rice bran was done in treatment 2-3 and 4-5, respectively. The objective of using corn starch is that corn starch contains very low amount of total phosphorus approximately 0.03%. Therefore, the increased levels of total phosphorus in the diets, as shown in Table 2-4, were due to the increased phytate phosphorus from defatted rice bran. Corn starch used in the experiment was in powder form and experimental diets were given to birds in mash form. Because of that, the control diet seemed to be dustier than other experimental diets

Table 21. Feed cost (dollar) per pound diet of different experimental diets.[1]

Diet	Treatment				
	1	2	3	4	5
Starter	0.18	0.17	0.17	0.14	0.15
Grower	0.17	0.15	0.15	0.13	0.13
Finisher	0.16	0.14	0.14	0.12	0.12

[1] Price of feed ingredients (dollar/pound): Corn, 0.102; Corn starch, 0.18; Defatted rice bran, 0.032; Soybean meal, 0.134; Corn gluten meal, 0.27; Fish meal, 0.35; Tallow, 0.21; Vitamin Mix, 0.80; Dicalcium phosphate, 0.19; Limestone, 0.05; Salt, 0.10; L-Lysine, 1.03; DL-Methionine, 3.60; Salinomycin, 0.89; Sand, 0.06; Phytase, 2.40.

Table 22. Feed cost per weight gain of broilers fed different experimental diets during 0-3 week of age.[1]

Treatment	Feed cost per weight gain 0-3 wk (dollars per pound)
1	0.40[a]
2	0.31[b]
3	0.31[b]
4	0.23[c]
5	0.23[c]
Pooled SEM	0.004
Probabilities	<0.0001

[1] Values are least square treatment means
[a, b, c] Means with different superscripts differ significantly (P<0.05)

Table 23. Feed cost per weight gain of broilers fed different experimental diets during 3-7 and 0-7 week of age under thermoneutral and heat stress conditions.[1]

Treatment	Temp.[2]	3-7 wk	0-7 wk
		----------(dollars per pound)----------	
1	TN	0.32	0.33
	HS	0.32	0.33
2	TN	0.29	0.29
	HS	0.28	0.28
3	TN	0.30	0.29
	HS	0.29	0.29
4	TN	0.23	0.23
	HS	0.23	0.23
5	TN	0.23	0.23
	HS	0.24	0.23
Pooled SEM		0.001	0.001
Source of Variation		----------Probabilities----------	
Diet		<0.0001	<0.0001
Temp.		0.4782	0.5118
Diet x Temp		0.3762	0.7571
Main Effect Means			
Diet			
1		0.32[a]	0.33[a]
2		0.29[b]	0.29[b]
3		0.29[b]	0.29[b]
4		0.23[c]	0.23[c]
5		0.24[c]	0.23[c]
Temp.			
TN		0.27	0.27
HS		0.27	0.27

[1] Values are least square treatment means
[2] TN = thermoneutral environment (23.9°C), HS = heat stress environment (23.9-35°C)
[a, b, c] Main effect means within columns with different superscripts differ significantly (P<0.05)

and appeared to be sticky inside the mouth of birds when diets were wet. Due to its low palatability, it consequently had an impact to decrease feed intake of birds during the last 4 weeks. The decreased feed intake contributed to the lowered body weight and weight gain of birds, and caused the birds in control group to have the least body weight and weight gain at 7-week of age (Table 7).

Because of low metabolizable energy of defatted rice bran compared with the other common energy sources such as corn, fat had to be added in diets containing defatted rice bran to adjust the overall metabolizable energy of the diets. The amount of fat added was increased as the level of defatted rice bran increased in the diets. In grower diets, high amounts of tallow were used in dietary treatment 4 and 5 (9.58 and 9.43%, respectively). Poultry are thought to regulate feed intake based on daily energy requirement. In theory, birds eat less high-energy feeds than low-energy feeds (Filmer, 2002). However, Coon (2001a,c) summarized from many studies that modern broilers, which have been selected for appetite, often consume as much feed of a high-energy diet and produce an increased output of gain. As energy intake increases, heat increment is reduced proportionally (MacLeod, 1990) and relative energy retention in the body occurs, usually as fat and protein (Leeson and Summers, 2001). In this experiment, birds may also have consumed more defatted rice bran diets compared to a control diet because of the increased palatability. High levels of fat in diets may lead to more metabolizable energy than the predicted energy value on the calculation basis from summation of ingredients (Mateos and Sell, 1980; Nitsan et al., 1997). Fat apparently caused the apparent metabolizable energy of these diets to be significantly higher than other diets

(Table 20). As a result, growth performance of birds fed defatted rice bran diets was found to be better than control diet, and higher level of defatted rice bran inclusion caused better performance. Although the activity of pancreatic enzymes increases rapidly posthatch, it appears that lipase activity is very limited in chicks during the first few weeks of age (Krogdahl, 1985; Denbow, 2000). However, broiler chicks could tolerate high level of dietary fat with the increase in lipase activity detected in the second week of age (Adrizal and Yayota, 2002). Hulan and Bird (1972) fed chicks isonitrogenous feeds in which the fat content increased from 4.5 to 14.5% and each feed was alternated over 4-day periods. The activities of amylase, protease, and lipase in the pancreatic juice were found to adjust with intake of the respective nutrients. The adaptations of lipid digestion to changes in diet composition were also summarized by Krogdahl (1985). In the current experiment, the abdominal fat percentage was also greater in birds fed higher level of defatted rice bran due to the direct incorporation of fat into the body as a part of growth.

The beneficial effect of supplemental fat in defatted rice bran diets was investigated in the study of Adrizal and Yayota (2002) conducted in broiler chicks from 1 to 14 days of age. Diets containing 30% defatted rice bran were supplemented with either fat (oil) or carbohydrate (soluble starch) in order to adjust the metabolizable energy of the diets. The nitrogen-corrected metabolizable energy (ME_n) value, protein and fat digestibilities were significantly greater with high-fat diets compared with high-carbohydrate diets. Those effects resulted in superior growth and feed intake, and increased feed conversion ratio of chicks similar to the results from the present experiment. Mateos and Sell (1980) suggested that the extra caloric effect of

supplemental fat may be due to an increase in intestinal retention time of feed which allowed for more complete digestion and absorption of nutrients. Bedford (2002) summarized research which showed that both the presence of an anticoccidial or added fat increase gain response in feed enzyme studies. Hormonal action is also involved in the increased digesta retention time of birds fed high fat diet. Cholecystokinin (CCK) is a hormone released from the duodenum and the jejunum in response to lipids and amino acids entering into small intestine (Denbow, 2000). Biological action of CCK is to inhibit gastric emptying and also to stimulate gall-bladder contraction, pancreatic enzyme secretion, and gastric acid secretion, which subsequently improves digestibility of nutrients and increases metabolizable energy of other feed constituents (Coon, 2001b). However, the effect on digesta retention time was not detected in this experiment. This may imply that there is an effect of defatted rice bran inclusion as well.

Water soluble non-starch polysaccharides that have the ability to cause high viscosity in the digestive tract can cause longer digesta retention time. Although there is more time that digesta stays in digestive tract, because of the increased viscosity there is less opportunity for substrates to interact with the enzymes (Smits and Annison, 1996). The reduced net energy was also observed by Choct (1999) that may be due to increased energy expended by the digestive system to move digesta through the system. Although the soluble isolate from rice bran was composed mainly of arabinoxylans similar to wheat, the ability to produce viscosity was less than wheat by approximately 40 fold due to different ratio of arabinose:xylose (Annison et al., 1995). Farrell and Martin (1998) found that relative gut viscosity also declined with increased rice bran inclusion (0, 20,

and 40%, respectively). Rice bran has been reported to have positive effects in human nutrition, such as laxative ability. That may be due to the water holding capacity of rice bran that shortens digesta retention time. Defatted rice bran has ability to bind water approximately five times its weight (Abdul-Hamid and Luan, 2000). Therefore non-starch polysaccharide in rice bran should not be an important limitation in the use of rice bran in poultry diets.

In this experiment, chicks fed 25% defatted rice bran diets without phytase supplementation had significantly higher weight and weight gain than chicks fed 25% defatted rice bran diet supplemented with phytase. This may be due to the ratio of male to female chicks in those treatments were not equal. Since the mixed sex day-old chicks were randomly assigned to each dietary treatment, it was possible that the ratio of male:female chicks in treatment 4 (25% defatted rice bran diet without phytase supplementation) was higher than that ratio in treatment 5 (25% defatted rice bran diet with phytase supplementation). Male chicks have higher growth rate than female chicks at the same age, therefore if the male:female ratio were equal, chicks in both treatment might have similar weight and weight gain. During 3-7 week of age, the difference between weight and weight gain of these two treatments were decreased because the ratio of male:female used in this period were equal.

Daily water intake and water:feed consumption ratio in the present experiment significantly increased as the level of defatted rice bran increased in the diets. In general, birds consume approximately twice as much water as the amount of feed consumed on a weight basis (NRC, 1994). Therefore, under normal conditions, water:feed consumption

ratio should range from about 1.5-2.5 (Bell, 2001). However, water intake can actually vary greatly. In this experiment, the ratio of water:feed consumption of birds on different experimental diets was found to be between 1.80-2.15 thus falling well within the expected range. Water intake is closely correlated with feed intake and factors affecting feed intake indirectly influence water consumption (Leeson and Summers, 2001). When the level of defatted rice bran was increased in the diets, birds consumed significantly more feed and consequently increased water intake.

Difference in environmental temperature significantly affected the feed intake of birds. Under heat stress condition, birds consumed significantly less feed than birds raised in the thermoneutral environment in order to limit extra heat production; consequently, birds gained less body weight. The study of Swain and Farrell (1975) showed a decline in heat production with increased temperature (varied from 5-34^{o}C) and with the duration of time that birds were exposed to those temperatures. Metabolizable energy concentration of the diet was found to increase gradually in birds exposed to 34^{o}C compared with birds exposed to 20^{o}C and diurnal 34/20^{o}C. However, the differences in apparent metabolizable energy were not detected in the present experiment.

Birds have no sweat glands, so evaporation of water from the skin is a natural mechanism for heat loss (Filmer, 2002). In fast growing birds, the surface area required for dissipation by conduction and convection increases only three fourths as fast as body weight (Teeter and Belay, 1996). Birds have to increase other physiological processes responsible for heat dissipation and lowering heat production. Filmer (2002) explained that birds needed to lose heat in order to maintain appetite. In hot and humid conditions

where birds could not lose enough heat, depressions in appetite and performance occurred. Teeter and Belay (1996) stated that heat production of birds exposed to heat stress condition is reduced largely as a result of lowered feed consumption. Birds can increase heat transfer to the body surface by reducing peripheral blood flow resistance and increasing resistance of blood flow to visceral organs (Bottje and Harrison, 1985). By reducing blood flow to the viscera, feed metabolism decreased and metabolic heat production was reduced (Wiernusz et al., 1992). When the difference between ambient temperature and bird's body temperature is reduced as ambient temperature rises, heat loss by way of non-evaporative cooling declines. Birds can dramatically increase evaporative cooling by increasing respiratory frequency as body temperature increased (Hillman et al., 1985). Water becomes extremely important in the regulation of body temperature. Heat loss due to evaporation of water from respiratory passageways can reach a maximum of approximately 100% at 35^{o}C (Farrell and Swain, 1977). The bird obtains most of its water by drinking. As environmental temperature arises, birds will drink more water in order to meet the demands for evaporative cooling. Under heat stress condition daily water intake of birds increased significantly in this experiment. In this study, because of the decline in feed consumption and the increase in water intake, water:feed consumption ratio was increased significantly.

Neither dietary nor temperature treatments significantly affected percent carcass weight of the birds. Percent abdominal fat increased as more fat was added into the diets. At high level of defatted rice bran inclusion, thighs percentage was increased significantly. This may due to an increase in protein digestibility and absorption.

However, the dietary treatment did not have an effect on percent breast in this experiment.

The composition of carcass was altered significantly because of heat stress. The percentage of breast was decreased under heat stress condition while the other market portions that had less protein content had higher percentages. Other researchers also found altered carcass composition under heat stress condition as well. Swain and Farrell (1975) reported that a significant increase in fat and a decrease in water content of carcasses were found in birds raised under high temperatures. Protein deposition was not significantly affected by temperature (varied from 5-34^{o}C) as measured by carcass analysis. However, there was a significant increase when estimated from nitrogen balance measurement.

Intestinal weight, weights of different parts of intestine, and morphology of duodenum and jejunum were not significantly affected by dietary or temperature treatments, except for percent ceca that was significantly decreased under heat stress condition. The decrease in cecal weight may imply a decrease in fermentation activity in ceca of those birds. An increase in the weight of small intestine and colon and the changes in morphology of intestinal lining of birds was found when birds fed diets containing high water soluble non-starch polysaccharide (Jorgensen et al., 1996; Smits et al., 1997; Iji, 1999). Since the non-starch polysaccharide in defatted rice bran does not have capacity to cause a viscous condition in digestive tract, it did not cause a significant difference in wet weight percentage of different parts of intestine or a change in duodenum and jejunum morphology in this experiment.

Very dramatic changes occur in relative organ weights to body weight ratio during development of the very young chick into mature laying hen, with various sections of the gastrointestinal track showing a decline from 2 to 20 weeks of age. Hens from 20 to 58 weeks of age demonstrated a trend of steady increase in weight of the large intestine and proventriculus, while the pancreas and gizzard weights were relatively stable (Sreemannarayana et al., 1989). The chick's intestinal system is anatomically complete early in embryonic development. The absorptive surface and rate of enterocyte proliferation increases after hatching by the presence of feed in gastrointestinal tract (Moran, 1985).

Not only can pancreatic zymogen levels in chicks adapt to optimize digestive need within the lumen (Hulan and Bird, 1972), but the absorptive surface also adapts to dietary conditions (Moran, 1985). *Gallus* species have villi that decrease in length from 1.5 mm in the duodenum to 0.4-0.6 mm in the ileum and rectum (Denbow, 2000). Genetic selection for growth has altered villi morphology (Yamauchi and Isshiki, 1991). The villi of broilers, compared to White Leghorn, are larger and show more epithelial cell protrusions from the apical surface of the duodenum villi. The villi from both types of chickens form a zig-zag arrangement, which is thought to slow ingesta flow. The increased villi height suggests an increased absorptive surface area capable of greater absorption of available nutrients (Caspary, 1992). A rapid turnover of the villus epithelium permits change in its length. Imondi and Bird (1966) reported that the chick's intestinal epithelium has a turnover time of approximately two days. The mechanism of the turnover has been explained by Moran (1985). It involves the generation of cells in

the crypt of Lieberkuhn at the base of each villus, which attains maturity while ascending the shaft. Cells are finally extruded from the tip of villi. Controlling the rates of division and extrusion, therefore, permits lengthening or shortening of the villi. The villi lengthening in animals fed *ad libitum* may be in response to a competition for nutrients with normal microflora (Cook and Bird, 1973). The adjustment in villi height is usually localized. For the most part, the upper jejunum of birds undergoes the greatest change because maximal digestion and presentation of nutrients for absorption occur through this area (Moran, 1985).

Sagher et al. (1991) reported an increase in villi height:crypt depth ratio in the jejunum and ileum of 3-week-old rats fed isoenergetic diets supplemental with 40% high-unsaturated fats (either olive oil or corn oil) compared to rats fed 40% high-saturated fat (butter) diet. Ritz et al. (1995) found an increase in villi height within the jejunum and ileum of young turkeys, at 2 and 3 weeks of age, fed corn-soybean diet supplemented with amylase. However, no responses were found in poults fed similar diet supplemented with xylanase. They concluded that this may be due to the low level of xylanase used in the experiment. In the current study, no differences were found in villi height, crypt depth or villi height:crypt dept. Since the chicks in this experiment were fed the dietary treatment from the first day of age until 7 weeks of age, the intestinal absorptive surface may have adapted in order to compensate for the high fiber in the diets.

The maintenance energy requirements of the liver and gastrointestinal tract represent a large portion of whole body maintenance energy requirement. This is partially associated with the high rates of cell turnover and protein synthesis in tissues of

gastrointestinal tract. Jin et al. (1994) reported the effect of dietary fiber on the rate of intestinal cell turnover as well as intestinal morphology in growing pigs. The high fiber diet increased crypt cell proliferation in jejunum and colon and increased cell death in jejunum and ileum. Depth of crypts was increased for jejunum, ileum, and colon. No responses were found in intestinal villi height, while width of villi was increased for jejunum and ileum. The non-starch polysaccharide also affected the changes in morphology of intestinal lining. The viscous environment in the intestinal lumen of chicks fed 3% pectin supplemented diet markedly reduced the number of zigzag patterns and ridge-shaped villi, whereas the number of tongue-shaped villi was higher (Langhout et al., 2000). However, no significant change in classification and shape of villi was observed in germ-free chicks fed the similar γ-irradiated diet. Therefore, the reduction in performance of conventional birds fed pectin supplemented diet may be due to a decrease in nutrient digestibility and the role of gastrointestinal microflora.

The effects of environmental temperature upon intestinal morphology and function have been shown in many species. The chronic exposure to elevated temperatures reduced the wet and dry weights of rodent's small intestine while it increasing the serosal transfer of glucose (Carpenter and Musacchia, 1978; Chu et al., 1979). The reduction in villi size was observed in the jejunum of piglets as the environmental temperature rose (Dauncey et al., 1983). Mitchell and Carlisle (1992) reported a reduction in size of the absorptive compartment in jejunum of growing broilers exposed to elevated environmental temperature, as reflected by decreased villi height (19%) as well as wet and dry weights per unit length of jejunum (26 and 31%,

respectively). *In vivo* jejunal uptakes of galactose and methionine were increased which may imply adaptation to optimize nutrient absorption when faced with reduced feed intake and decreases in size of the absorptive compartment. Although morphological changes are often associated with intestinal adaptation, the increase in absorption ability does not necessarily correlate with hyperplasia (Woudstra and Thomson, 2002). In a study of rat jejunum, Thomson et al. (1986) found that the uptake of glucose is enhanced by dietary fats but is not associated with increased mucosal surface area. In the current study, however, there was no difference between two temperature treatments.

The primary objective of using phytase in diets is to increase hydrolysis of phytate that is abundant in plant feed ingredients but could not be hydrolyzed by endogenous enzyme of animals, and also to decrease phosphorus loss in excreta. Phytase does not only increase phytate phosphorus utilization by 20-45% (Ravindran et al., 1995), but also releases other minerals that are complexed with phytate. An increased bioavailability of essential dietary minerals may also be involved with the performance of birds. Although from the summary of many studies on effect of microbial phytase on broiler performance, it was concluded that supplemental phytase improved feed intake and feed conversion ratio of broilers (Rosen, 2002), those effects were not detected in this experiment.

In this experiment, percent tibia ash as a measure of mineral content was found to be significantly affected by increased defatted rice bran inclusion. Inclusion of 10% defatted rice bran in diets did not significantly affect percent ash, calcium:phosphorus ratio, or zinc content, but higher levels of defatted rice bran did. In 3-week old chicks,

tibia phosphorus was increased while percent calcium was decreased when higher level of defatted rice bran was included in diets, therefore the ratio of calcium:phosphorus was decreased. The supplementation of phytase combined with the decrease in available phosphorus by 0.1% in the diet altered the calcium:phosphorus ratio. Since there was an increase in percent tibia phosphorus of 3-week old birds, calcium:phosphorus ratio was significantly decreased. At high levels of defatted rice bran, the level of phytase used (500 units/kg) may be too low, therefore no significant differences were found. As the birds aged, the ability to utilize calcium may be higher. Therefore, no difference among dietary treatments was found in percent calcium of 7-week old birds and the ratio of calcium:phosphorus was increased with increased defatted rice bran inclusion. The effect of phytase in increasing percent tibia phosphorus was found only at the high level of defatted rice bran inclusion.

Zinc and manganese can form complexes with phytate in which zinc has the most stable complexes (Cheryan et al., 1983). In the experiment reported here, increasing levels of defatted rice bran did not affect the zinc content in tibia. Manganese content significantly decreased when defatted rice bran was incorporated in the diets. This was inconsistent with the study of Halpin and Baker (1986) who reported different results. In that study, the 10% rice bran-casein-dextrose diets were calculated to have 3 different levels of inorganic manganese (0, 7, 14 mg/kg). Rice bran supplementation increased tissue manganese concentrations at all three levels of supplemental inorganic manganese, and the greater responses occurred when the levels of manganese were at or below the chick's requirement.

Phytase significantly affected zinc and manganese content in tibia only at low levels of defatted rice bran in 3-week-old chicks. This again might be due to the low level of phytase used in this experiment. In ducks, Farrell and Martin (1993) found that tibia ash showed a response to phytase (1000 units/kg) when 0 and 30% rice bran were included in diets, but not at 60% inclusion.

Leske and Coon (1999) determined the effect of phytase when using defatted rice bran as the sole source of phosphorus. In broilers, phytate phosphorus hydrolysis and total phosphorus retention improved by approximately 14.8 and 11.0%, while those values were approximately 14.8 and 7.1% in layers, respectively. In this experiment, as expected, high levels of defatted rice bran in diets caused an increase in total phosphorus in the excreta, which in turn caused a decrease in phosphorus retention. Percent calcium in excreta was decreased when high level of defatted rice bran in diets was used. However, due to the increased amount of excreta, calcium retention was also decreased. Although there was a decrease in excreta phosphorus when phytase was added to the defatted rice bran diets, the level of phytase used in this experiment did not significantly affect calcium or phosphorus in excreta nor did it improve their retentions.

The price of defatted rice bran used in this experiment was cheaper than other major feed ingredients and the price of corn starch was quite high, therefore feed cost per kg diet was lower as level of defatted rice bran increased. Because of higher weight gain and better feed conversion ratio, feed cost per weight gain of chicks fed higher level of defatted rice bran inclusion was lower. Adding phytase did not affect growth performance of birds and the level of phytase used in diets was very low, therefore it did

not affect feed cost per weight gain. The difference in environmental temperature also did not have an impact on feed cost per gain.

Conclusion

Using up to 25% defatted rice bran in broiler diets with fat added to adjust the metabolizable energy, did not show any adverse effect on growth performance of broilers. The beneficial effect of high fat level could overcome the problem associated with high phytate phosphorus in defatted rice bran. The non-starch polysaccharide in defatted rice bran did not seem to be an important factor when using defatted rice bran up to 25% in diets. Using phytase 500 units/kg in diets with 0.1% decrease in available phosphorus, did not affect the performance of birds, but helped to improve the phosphorus, zinc, and manganese content in tibia of chicks fed low level of defatted rice bran. Although phosphorus in the excreta was decreased when using phytase in defatted rice bran diets, phosphorus retention of birds was not significantly improved. Adverse effects of heat stress on growth performance could not be improved by phytase supplementation.

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PART IV

EXPERIMENT 2: BROILER PERFORMANCE AND MINERAL UTILIZATION OF PHYTASE AND XYLANASE-SUPPLEMENTED DEFATTED RICE BRAN DIET DURING HEAT STRESS

Abstract

An experiment was conducted to determine broiler performance and mineral utilization of defatted rice bran diets supplemented with phytase and xylanase under two different environmental temperatures. Five replicate groups of chicks were assigned to six dietary treatments. There were two control diets with the first one being a corn-soybean meal based diet, while the other one was supplemented with 25% corn starch, and the other four diets containing two levels of defatted rice bran (10 and 25%) with or without both commercial phytase (500 units/kg diet) and xylanase (1000 units/kg diet) supplementation. Available phosphorus in diets containing phytase was calculated to be 0.1% reduction from the requirement for broilers. On day 21, some birds were slaughtered and tibias harvested for mineral analysis. The remaining birds were transferred to either a thermoneutral temperature chamber (23.9^{o}C) or a heat stress chamber (23.9-35^{o}C, diurnal). Digesta retention time was determined and a 5-day total excreta collection was done on 35-day of age. Birds were slaughtered at seven weeks of age, tibias were harvested, and intestines examined. Birds fed 25% defatted rice bran diet had the highest body weight gain. Supplemental enzyme improved feed conversion ratio of chicks fed 10% defatted rice bran diet. No significant differences were found in most parts of the carcass and morphology of the duodenum and the jejunum. Tibia ash and zinc content were not affected by dietary treatment. Tibia calcium, phosphorus, and manganese content of 3-week-old chicks were improved when enzyme was added to 25% defatted rice bran diet. However, only the improvement of percent tibia phosphorus of 7-week-old birds were detected when enzyme was added in 10% defatted rice bran diet.

Phosphorus retention increased almost 100% in birds fed 25% defatted rice bran diet supplemented with enzyme. However, it was still very low. Feed cost per weight gain of birds fed 25% defatted rice bran diet without enzyme supplementation was lowest. From the growth performance and feed cost per weight of birds, supplemental enzyme was not beneficial in heat stress environment. Using high fat to adjust the metabolizable energy of defatted rice bran diets seemed to enhance growth therefore the benefit of added xylanase was not detected.

Introduction

Rice bran contains relatively high level of fiber and phytate that monogastric animals are unable to digest. If the problem elements can be broken down, the animal has greater access to the available nutrients, thereby overcoming the negative impacts of incorporating rice bran in the diet. Rice bran contains approximately 9.8-15.4% protein with 7.7-22.4% fat (Houston, 1972). The fat in bran contains a high concentration of unsaturated fatty acids (80-85%), which becomes rapidly rancid if it is stored improperly. After extracting oil from rice bran, defatted rice bran can be used as an animal feed ingredient. The utilization of rice bran in diets of poultry has been reviewed by Farrell (1994).

Defatted rice bran contains over 70% of non-starch polysaccharides mainly arabinose and xylose (Annison et al., 1995). High levels of non-starch polysaccharide, especially the water-soluble part, can interfere with the digestion and absorption of

nutrients in digestive tract of broilers. Abnormal intestinal absorptive surface and activity were observed in rats and broiler chicks and this may contribute to the poor absorption of nutrients (Pell et al., 1992; Langhout et al., 1999, 2000). Defatted rice bran is also high in phytate phosphorus with this form constituting approximately 90% of total phosphorus (Juliano, 1985). Phytate is a polyanionic molecule with six phosphate groups, which can readily form insoluble complexes with many divalent cations, such as Zn^{2+}, Cu^{2+}, Ni^{2+}, Co^{2+}, Mn^{2+}, Ca^{2+}, and Fe^{2+}, in weak acidic to neutral pH conditions and, consequently, reduces their bioavailability in animals (Davies and Nightingale, 1975; Cheryan et al., 1983). Halpin and Baker (1987) found that both neutral detergent fiber and ash fractions of rice bran significantly lowered tissue manganese concentrations, but not to the same extent as that caused by rice bran itself. This result implies that both phytate and non-starch polysaccharide in rice bran can decrease bioavailability of minerals in diets. Both non-starch polysaccharide and phytate are indigestible by simple stomach animals (Scott et al., 1982).

Enzymes in feed industry have mostly been used for poultry and pigs to neutralize the effects of the viscous, non-starch polysaccharides in cereals such as barley, wheat, and rye (Bedford and Morgan, 1996). Enzymes allow the use of a wide range of ingredients without compromising bird performance and hence provide great flexibility in least-cost feed formulation. The significant improvements in feed consumption, weight gain, and feed conversion ratio were found in Leghorn chicks fed wheat based diet containing 40% rice bran and corn-based diet containing 25% rice bran supplemented with xylanase (Wang et al., 1995).

The use of exogenous phytase as a feed additive also has been increasing recently to improve the bioavailability of phosphate and other minerals in plant feed ingredients. The concern about environmental pollution from the excess phytate in farm animal manure used in agricultural fields also leads to the increase use of phytase as a feed additive. Leske and Coon (1999) found that the addition of phytase significantly increased phosphorus hydrolysis and total phosphorus retention of defatted rice bran in broilers and laying hens.

Under elevated ambient temperature conditions, birds consume less feed in order to reduce metabolic heat production. Birds will consume more water to fulfill demands for evaporative cooling by panting. These birds gain less body weight and survivability is reduced (Dale and Fuller, 1980; Teeter and Belay, 1996). The availability of manganese, zinc, and other minerals is reduced due to increased excretion (Belay and Teeter, 1991). Addition of commercial phytase to defatted rice bran diets may improve the mineral retention of birds fed defatted rice bran diet, especially in heat-distressed broilers. Together with supplemental commercial xylanase, the growth performance of birds fed defatted rice bran may be improved. Therefore, the purposes of this experiment were to:

- Evaluate the production performance of broilers fed defatted rice bran diets supplemented with commercial phytase and xylanase under two different environmental temperatures.

- Evaluate the potential benefits of using commercial phytase and xylanase to improve phosphorus retention, decrease phosphorus excretion, and improve

bioavailability of phosphorus, calcium, zinc, and manganese of birds fed defatted rice bran diets.

Materials and Methods

Chickens and Diets

The procedures used in the animal work were approved by the University of Tennessee Institutional Animal Care and Use Committee. Four-hundred and twenty day-old mixed sex commercial broilers (Ross x Ross) were used in this experiment. They were distributed among thirty pens in a single brooder house. Five replicate groups of fourteen chicks were randomly assigned to each of six dietary treatments:

Diet 1: control diet without defatted rice bran

Diet 2: control diet with 25% corn starch, without defatted rice bran

Diet 3: 15% corn starch, 10% defatted rice bran

Diet 4: 15% corn starch, 10% defatted rice bran + phytase and xylanase

Diet 5: 25% defatted rice bran

Diet 6: 25% defatted rice bran + phytase and xylanase

Diets were formulated to meet or exceed current National Research Council recommendations (NRC, 1994). The compositions of experimental diets are shown in Table 1, 2, and 3. There were two control diets with the first being a practical corn-soybean meal based diet, while the other one was supplemented with 25% corn starch.

Table 1. Composition of starter diets (%).

Treatment	1	2	3	4	5	6
Corn	64.48	33.38	34.26	34.74	33.52	36.02
Corn starch	-	25.00	15.00	15.00	-	-
Defatted rice bran	-	-	10.00	10.00	25.00	25.00
Soybean meal	14.35	19.66	16.17	16.08	14.75	10.89
Corn gluten meal	10.00	10.00	10.00	10.00	9.38	9.98
Fish meal	7.00	7.00	7.00	7.00	4.85	7.00
Tallow	0.23	1.08	4.00	3.84	9.14	8.23
Vitamin Mix[1]	0.50	0.50	0.50	0.50	0.50	0.50
Dicalcium phosphate	1.89	1.96	1.88	1.34	1.73	1.22
Limestone	0.46	0.40	0.13	0.44	-	0.03
Salt	0.30	0.30	0.30	0.30	0.30	0.30
L-Lysine	0.11	0.02	0.07	0.07	0.14	0.15
DL-Methionine	0.08	0.10	0.09	0.09	0.09	0.08
Salinomycin	0.10	0.10	0.10	0.10	0.10	0.10
Sand	0.50	0.50	0.50	0.35	0.50	0.35
Phytase[2]	-	-	-	0.05	-	0.05
Xylanase[3]	-	-	-	0.10	-	0.10
Total	100	100	100	100	100	100
Calculated nutrient composition						
Crude protein, %	23.00	23.00	23.00	23.00	23.00	23.00
ME, kcal/kg	3200	3200	3200	3200	3200	3200
Calcium, %	1.00	1.00	1.00	1.00	1.00	1.00
Available phosphorus, %	0.45	0.45	0.45	0.35	0.45	0.35
Total phosphorus, %	0.87	0.84	0.98	0.88	1.13	1.08
Zinc, ppm	101.1	98.50	99.66	99.16	99.73	100.9
Manganese, ppm	118.2	125.7	146.2	144.6	177.5	175.2
Lysine, %	1.10	1.10	1.10	1.10	1.10	1.10
Methionine, %	0.55	0.55	0.55	0.55	0.54	0.54

[1] Supplied per kilogram of diet: Copper, 8.8 mg; Iodine, 1.1 mg; Iron, 22 mg; Manganese, 99 mg; Selenium, 0.17 mg; Zinc, 66 mg; Vitamin A, 14960 IU; Vitamin D_3, 5060 IU; Vitamin E, 77 IU; Vitamin B_{12}, 0.02 mg; Vitamin K_3, 4.4 mg; Biotin, 0.22 mg; Choline, 1100 mg; Folic acid, 2.2 mg; Niacin, 77 mg; d-Pantothenic acid, 17.6 mg; Vitamin B_6, 6.6 mg; Riboflavin, 8.8 mg, Thiamine, 3.3 mg.

[2] Allzyme SSF Phytase, provided by Alltech, Nicholasville, Kentucky, contains 1000 PU/g

[3] Allzyme PT, provided by Alltech, Nicholasville, Kentucky, contains 1000 XU/g

Table 2. Composition of grower diets (%).

Treatment	1	2	3	4	5	6
Corn	70.82	41.32	42.127	42.657	39.69	40.20
Corn starch	-	25.00	15.00	15.00	-	-
Defatted rice bran	-	-	10.00	10.00	25.00	25.00
Soybean meal	13.74	15.86	12.54	12.37	14.60	14.44
Corn gluten meal	7.14	10.00	9.18	9.16	4.94	4.93
Fish meal	4.54	3.76	4.54	4.62	3.78	3.86
Tallow	-	0.13	3.12	2.94	9.06	8.88
Vitamin Mix[1]	0.50	0.50	0.50	0.50	0.50	0.50
Dicalcium phosphate	1.35	1.43	1.36	0.82	1.20	0.66
Limestone	0.84	0.90	0.52	0.82	0.18	0.48
Salt	0.30	0.30	0.30	0.30	0.30	0.30
L-Lysine	0.17	0.19	0.21	0.21	0.12	0.12
DL-Methionine	-	0.01	0.003	0.003	0.03	0.03
Salinomycin	0.10	0.10	0.10	0.10	0.10	0.10
Sand	0.50	0.50	0.50	0.35	0.50	0.35
Phytase[2]	-	-	-	0.05	-	0.05
Xylanase[3]	-	-	-	0.10	-	0.10
Total	100	100	100	100	100	100
Calculated nutrient composition						
Crude protein, %	20.00	20.00	20.00	20.00	20.00	20.00
ME, kcal/kg	3200	3200	3200	3200	3200	3200
Calcium, %	0.90	0.90	0.90	0.90	0.90	0.90
Available phosphorus, %	0.35	0.35	0.35	0.25	0.35	0.25
Total phosphorus, %	0.70	0.65	0.80	0.71	0.99	0.89
Zinc, ppm	96.82	92.55	94.67	94.25	97.19	96.77
Manganese, ppm	115.9	122.0	142.8	141.1	175.8	174.1
Lysine, %	1.00	1.00	1.00	1.00	1.00	1.00
Methionine, %	0.40	0.40	0.40	0.40	0.40	0.40

[1] Supplied per kilogram of diet: Copper, 8.8 mg; Iodine, 1.1 mg; Iron, 22 mg; Manganese, 99 mg; Selenium, 0.17 mg; Zinc, 66 mg; Vitamin A, 14960 IU; Vitamin D_3, 5060 IU; Vitamin E, 77 IU; Vitamin B_{12}, 0.02 mg; Vitamin K_3, 4.4 mg; Biotin, 0.22 mg; Choline, 1100 mg; Folic acid, 2.2 mg; Niacin, 77 mg; d-Pantothenic acid, 17.6 mg; Vitamin B_6, 6.6 mg; Riboflavin, 8.8 mg, Thiamine, 3.3 mg.

[2] Allzyme SSF Phytase, provided by Alltech, Nicholasville, Kentucky, contains 1000 PU/g

[3] Allzyme PT, provided by Alltech, Nicholasville, Kentucky, contains 1000 XU/g

Table 3. Composition of finisher diets (%).

Treatment	1	2	3	4	5	6
Corn	74.09	45.15	45.29	45.72	43.68	44.78
Corn starch	-	25.00	15.00	15.00	-	-
Defatted rice bran	-	-	10.00	10.00	25.00	25.00
Soybean meal	14.55	15.76	13.75	13.73	14.21	12.94
Corn gluten meal	5.16	7.02	5.37	5.33	1.60	1.76
Fish meal	2.59	3.59	4.31	4.31	4.29	5.00
Tallow	-	-	3.27	3.12	8.88	8.48
Vitamin Mix[1]	0.50	0.50	0.50	0.50	0.50	0.50
Dicalcium phosphate	1.08	1.17	1.09	0.56	0.94	0.42
Limestone	0.99	0.81	0.43	0.74	-	0.22
Salt	0.30	0.30	0.30	0.30	0.30	0.30
L-Lysine	0.14	0.10	0.09	0.09	-	-
Salinomycin	0.10	0.10	0.10	0.10	0.10	0.10
Sand	0.50	0.50	0.50	0.35	0.50	0.35
Phytase[2]	-	-	-	0.05	-	0.05
Xylanase[3]	-	-	-	0.10	-	0.10
Total	100	100	100	100	100	100
Calculated nutrient composition						
Crude protein, %	18.23	18.24	18.23	18.23	18.24	18.24
ME, kcal/kg	3200	3200	3200	3200	3200	3200
Calcium, %	0.80	0.80	0.80	0.80	0.80	0.80
Available phosphorus, %	0.30	0.30	0.30	0.20	0.30	0.20
Total phosphorus, %	0.60	0.59	0.74	0.65	0.95	0.87
Zinc, ppm	94.06	91.70	94.05	93.56	97.09	97.16
Manganese, ppm	114.9	121.3	142.5	140.9	175.1	173.3
Lysine, %	0.90	0.90	0.90	0.90	0.89	0.89
Methionine, %	0.35	0.35	0.35	0.35	0.33	0.34

[1] Supplied per kilogram of diet: Copper, 8.8 mg; Iodine, 1.1 mg; Iron, 22 mg; Manganese, 99 mg; Selenium, 0.17 mg; Zinc, 66 mg; Vitamin A, 14960 IU; Vitamin D_3, 5060 IU; Vitamin E, 77 IU; Vitamin B_{12}, 0.02 mg; Vitamin K_3, 4.4 mg; Biotin, 0.22 mg; Choline, 1100 mg; Folic acid, 2.2 mg; Niacin, 77 mg; d-Pantothenic acid, 17.6 mg; Vitamin B_6, 6.6 mg; Riboflavin, 8.8 mg, Thiamine, 3.3 mg.

[2] Allzyme SSF Phytase, provided by Alltech, Nicholasville, Kentucky, contains 1000 PU/g

[3] Allzyme PT, provided by Alltech, Nicholasville, Kentucky, contains 1000 XU/g

The substitution of corn starch with 10 and 25% defatted rice bran was done in treatment 3-4 and 5-6, respectively. The increased levels of total phosphorus in the diets were due to the increased phytate phosphorus from defatted rice bran. Tallow was used as the dietary fat source. Phytase (Allzyme SSF Phytase, Alltech Inc.) containing 1000 units of phytase per gram, was used at 0.05% in the diet. Xylanase (Allzyme PT, Alltech Inc.) containing 1000 units of xylanase per gram, was used at 0.10% in the diet. The level of available phosphorus in all diets containing phytase was reduced by 0.10%: from 0.45% to 0.35% in starter period (0-3 week of age), 0.35% to 0.25% in grower period (3-6 week of age), and 0.30% to 0.20% in finisher period (6-7 week of age), respectively. Analyzed nutrient composition of the experimental diets is shown in Table 4. All diets were fed in mash form. Chicks were weighed and allowed *ad libitum* access to feed and water. During the first 21 days posthatch, birds were kept under 23 hours light:1 hour dark photoperiod and thermoneutral condition. Weekly feed consumption and body weight were recorded.

Temperature Treatments

On day 22, eight birds from each replicate were randomly selected and weighed individually. They were divided into two groups and transferred to individual metabolism cages in two environmental chambers, maintaining the same dietary treatments for the next four weeks (Table 2 and 3). The ambient temperature in one chamber was controlled to be constant at 23.9^{o}C (thermoneutral) while the other one was allowed to cycle between 23.9 and 35^{o}C (heat stress) in order to simulate diurnal

Table 4. Analyzed nutrient composition of experimental diets (%).

Starter diets	Treatment					
	1	2	3	4	5	6
Crude protein, %	26.18	26.91	25.67	26.67	25.30	24.62
Fat, %	3.93	3.77	7.59	7.14	12.90	12.17
Ash, %	8.82	7.51	7.88	7.52	8.44	6.92
Calcium, %	1.46	1.01	0.95	1.12	0.92	0.88
Total phosphorus, %	1.06	0.86	0.99	1.09	1.09	1.03
Grower diets	Treatment					
	1	2	3	4	5	6
Crude protein, %	24.64	22.05	23.11	23.99	22.61	23.03
Fat, %	2.26	2.81	5.68	6.71	12.09	11.54
Ash, %	8.18	5.71	6.87	6.06	8.08	7.37
Calcium, %	1.23	0.91	0.93	0.94	0.90	0.92
Total phosphorus, %	0.89	1.09	0.98	1.09	1.06	0.90
Finisher diets	Treatment					
	1	2	3	4	5	6
Crude protein, %	21.04	20.89	21.15	20.95	20.73	20.49
Fat, %	2.42	1.87	6.76	7.46	13.01	13.12
Ash, %	5.79	5.28	6.77	5.61	7.71	7.13
Calcium, %	0.87	0.88	0.87	0.92	0.75	0.81
Total phosphorus, %	0.59	0.89	0.95	0.89	1.08	0.99

temperature pattern. The photoperiod was held at 23 hours light:1 hour dark for both chambers. Feed consumption, water consumption, and body weight were monitored on a weekly basis. The remaining birds were humanely slaughtered and both tibias of three birds from each replicate were harvested and frozen for calcium, phosphorus, zinc, and manganese analysis.

Digesta Retention Time Measurement and Excreta Collection

On day 35, following a 12-hour fast, birds were allowed to consume 30 g ferric oxide labeled feed (1.5% ferric oxide) as an indigestible marker following which the time of first appearance of a bright red tint of ferric oxide in the feces was noted as a measure of digesta retention time. Two male and two female birds from each treatment within each chamber were used for excreta collection. Individual stainless steel trays were placed under each cage for excreta collection. The amount of feed intake and excreta were recorded and all of the excreta was collected for 5 consecutive days. Ferric oxide labeled feed was again given on the fifth day and excreta collection ceased when a bright red tint of ferric oxide appeared following the second dosing. The collected excreta was frozen for analysis of calcium and phosphorus and apparent metabolizable energy determination.

Processing of Birds

On day 49, following a 12-hour fast, five male and five female birds from each treatment within each chamber were humanely slaughtered. The carcasses were then manually eviscerated. The whole intestines were carefully pulled out. Abdominal fat

pad (including fat surrounding gizzard, bursa of Fabricius, cloaca, and adjacent muscles) was weighed individually and calculated as a percentage of live weight of the bird. Following evisceration, each part of intestine of two male and two female birds from each treatment within each chamber were separated; the intestinal segment from the gizzard to pancreatic and bile ducts as duodenum, jejunum from the ducts to Meckel's diverticulum, and ileum from the diverticulum to the ileo-cecal-colonic junction. After removal of residual digesta by manual compression, the weights of duodenum, jejunum, ileum, ceca, and colon were determined. Each part of intestine was calculated as a percentage of the whole intestinal weight, and the whole intestinal weight was calculated as a percentage of live weight of the bird. Duodenum and jejunum of the rest of the birds were carefully collected for villi height and crypt depth measurements. Each carcass was weighed, separated into market portions (breast, thigh, legs, wings, back) and each part weighed. The parts yield was calculated as a percentage of the whole carcass. During the slaughtering process, the right tibia was harvested and frozen for calcium, phosphorus, zinc, and manganese analysis.

Villi Height and Crypt Depth Measurements

The duodenum and jejunum of three male and three female birds from each treatment within each chamber collected during the slaughtering process were carefully separated. The middle part of each duodenum and jejunum segment was cut about 6 centimeters long. Each portion was cut again half way along the side, opened up, and rinsed lightly inside and outside with buffered formalin. Each sample was stored at room

temperature in 30 ml buffered formalin. Later, slide sections were prepared by paraffin embedding the sample, sectioning, and staining with hematoxylin-eosin.

The slide of each sample was studied under an automated microscope. The photographs of at least 10 villi per slide, showing complete section of villi and crypt, were taken by a digital camera connected to a computerized MetaMorph Imaging System. The system measured villi height and crypt depth by calculating a distance along the line from the villus tip to the bottom and from the bottom of the villus to the deepest part of crypt, respectively. The mean villi height and crypt depth of duodenum and jejunum of each bird were used for statistical analysis.

Tibia Mineral Determination

Tibias of birds harvested at 3-week and 7-week of age were prepared for calcium, phosphorus, zinc, and manganese analysis. All adhering tissue was removed from the bones. Two tibias from the same 3-week-old birds and a tibia from 7-week-old birds were cracked and wrapped individually with cheesecloth before defatting with petroleum ether. After drying at 60^{o}C for 12 hours, bones were removed from cheesecloth, placed into crucibles and then ashed in a muffle furnace at 600^{o}C for 12 hours. Bone ash was ground and about 1 gram solubilized in 3 ml of 6 N hydrochloric acid before further diluting with deionized water. Analysis of calcium, zinc, and manganese were done using a Unicam Solaar 969 atomic absorption spectrometer, while colorimetrical analysis of phosphorus was done using a BioMate 5 spectrometer.

Calcium and Phosphorus Retention Determination

The collected excreta was defrosted at room temperature, pooled, dried at 50°C, weighed, and ground. Excreta samples and grower diet samples were weighed and about 1 gram/sample digested with hydrochloric acid before further diluting with deionized water. Analysis of calcium was done using a Unicam Solaar 969 atomic absorption spectrometer, while colorimetrical analysis of total phosphorus was done using a BioMate 5 spectrometer. Calcium retention was calculated during 5-day excreta collection as follows:

$$\frac{\text{Total calcium in feed} - \text{Total calcium in excreta}}{\text{Total calcium in feed}} \times 100$$

Phosphorus retention was calculated similarly.

Apparent Metabolizable Energy Determination

After drying at 50°C and grinding, gross energy of excreta samples as well as grower diet samples were determined using an oxygen bomb calorimeter. Apparent metabolizable energy was calculated during 5-day excreta collection as total gross energy of feed minus total gross energy of excreta.

Feed Cost per Weight Gain Determination

Price of feed ingredients used in each feed formulation was used for calculation of feed cost. Feed costs per weight gain production of birds in each replicate during 0-3 week of age were calculated as feed costs of starter diets multiplied by feed conversion

ratio of birds in each replicate during 0-3 week of age. Feed cost per weight gain of each bird during 3-7 week of age was calculated as follows:

$$\frac{[(\text{Feed intake 3-6 wk}) \times (\text{Grower diet cost})] + [(\text{Feed intake 6-7 wk}) \times (\text{Finisher diet cost})]}{(\text{Weight gain 3-7 wk})}$$

Finally, feed cost per weight gain of each bird during 0-7 week of age was calculated by adding [(average of feed intake 0-3 week of each treatment) **x** (cost of starter diet)] to the equation above, then dividing by [(average weight gain 0-3 week of each treatment) + (weight gain 3-7 week)].

Experimental Design and Statistical Analysis

Analysis of variance was performed on all data using the mixed model analysis of SAS software (mixed procedure of SAS, 2001). For the first 21 days, the pens were used as replicates. For the heat stress portion of the study, each bird was used as a replicate. Least square treatment means were compared and differences were considered significant at the 5% level of probability.

Results

Growth Performance

Average body weight, weight gain, feed intake, and feed conversion ratio of 3-week-old broilers are shown in Table 5. Significant differences were found in body weight and weight gain ($P<0.01$), where chicks fed 25% corn starch diet had the lowest

Table 5. Body weight, weight gain, feed intake, and feed conversion ratio of 3-week-old broilers fed different experimental diets.[1]

Treatment[2]	Body weight (g)	Weight gain (g)	Feed intake (g)	Feed conversion ratio
1	573.79^{d}	527.05^{d}	930.52^{b}	1.76^{bc}
2	502.02^{e}	455.33^{e}	903.62^{b}	1.98^{a}
3	582.02^{cd}	534.93^{cd}	1010.06^{a}	1.88^{ab}
4	616.27^{bc}	569.20^{bc}	920.65^{b}	1.61^{cd}
5	707.54^{a}	660.39^{a}	999.21^{a}	1.51^{d}
6	624.67^{b}	577.90^{b}	1006.78^{a}	1.74^{bc}
Pooled SEM	4.42	4.41	8.38	0.02
Probabilities	<0.0001	<0.0001	0.0049	<0.0001

[1] Values are least square treatment means
[2] Treatment 1 = basal diet: corn-soybean diet
Treatment 2 = basal diet: 25% corn starch
Treatment 3 = diet with 15% corn starch, 10% defatted rice bran
Treatment 4 = diet with 15% corn starch, 10% defatted rice bran + phytase and xylanase
Treatment 5 = diet with 25% defatted rice bran
Treatment 6 = diet with 25% defatted rice bran + phytase and xylanase
[a, b, c, d, e] Means within columns with different superscripts differ significantly ($P<0.05$)

body weight and weight gain. Chicks fed corn-soybean diet had significantly higher body weight and weight gain, the same level as birds fed 10% defatted rice bran diet without enzymes supplementation. Chicks fed 25% defatted rice bran diets without enzyme supplementation had the highest weight, which was significantly higher than chicks fed the same level of defatted rice bran diet supplemented with enzymes. Adding enzymes to the 10% defatted rice bran diet improved body weight and weight gain of chicks but the differences were not significant.

Feed intake of chicks fed defatted rice bran diets was higher than that of chicks on the control diets ($P<0.01$). Enzyme supplementation significantly lowered feed intake of birds fed 10% defatted rice bran diet to the same level as control diets but did not impact birds fed 25% defatted rice bran diet. Birds fed the 25% corn starch diet had the worst feed conversion ratio ($P<0.01$), but it was not different from that of birds fed 10% defatted rice bran diet without enzymes supplementation. Birds fed 25% defatted rice bran diet without enzymes supplementation had the best feed conversion ratio. Adding enzymes in the diets helped to significantly lower feed conversion ratio of birds fed 10% defatted rice bran diet, but significantly increased feed conversion ratio of birds fed 25% defatted rice bran diet.

The growth performance of birds for the period in the temperature-controlled chambers is shown in Table 6. The total weight gain, feed intake, and feed conversion ratio of broilers during 0-7 week of age are shown in Table 7 and Figure 1. The growth performance of birds in both periods were significantly affected by dietary treatments

Table 6. Body weight at 7 week of age, weight gain, feed intake, and feed conversion ratio during 3-7 week of age of broilers fed different experimental diets under thermoneutral and heat stress conditions.[1]

Treatment	Temp.[2]	Body weight (g)	Weight gain (g)	Feed intake (g)	Feed conversion ratio
1	TN	2397.02	1772.10	3768.83	1.98
	HS	1887.51	1236.43	2682.60	2.14
2	TN	2139.33	1567.81	2906.79	1.86
	HS	1669.05	1103.12	2200.48	1.98
3	TN	2423.42	1792.10	3247.17	1.82
	HS	1940.29	1281.51	2358.27	1.85
4	TN	2399.72	1692.54	3243.41	1.93
	HS	1907.98	1214.80	2333.40	1.93
5	TN	2696.50	1945.56	3390.80	1.79
	HS	1977.29	1233.37	2297.54	1.87
6	TN	2604.01	1901.57	3297.65	1.74
	HS	2079.75	1373.32	2460.34	1.80
Pooled SEM		13.55	11.95	22.60	0.01
Source of Variation		----------Probabilities----------			
Diet		0.0001	0.0007	0.0006	<0.0001
Temp.		<0.0001	<0.0001	<0.0001	0.0020
Diet x Temp		0.3235	0.1863	0.3220	0.2255
Main Effect Means					
Diet					
1		2142.26^{b}	1504.26^{bc}	3225.72^{a}	2.06^{a}
2		1904.19^{c}	1335.47^{d}	2553.64^{c}	1.92^{b}
3		2181.85^{b}	1536.81^{abc}	2802.72^{b}	1.83^{c}
4		2153.85^{b}	1453.67^{c}	2788.41^{b}	1.93^{b}
5		2336.89^{a}	1589.47^{ab}	2844.17^{b}	1.83^{c}
6		2341.88^{a}	1637.44^{a}	2878.99^{b}	1.77^{c}
Temp.					
TN		2443.33^{a}	1778.61^{a}	3309.11^{a}	1.85^{b}
HS		1910.31^{b}	1240.42^{b}	2388.77^{b}	1.93^{a}

[1] Values are least square treatment means

[2] TN = thermoneutral environment (23.9^{o}C), HS = heat stress environment (23.9-35^{o}C)

[a, b, c] Main effect means within columns with different superscripts differ significantly (P<0.05)

Table 7. Total weight gain, feed intake, and feed conversion ratio during 0-7 week of age of broilers fed different experimental diets under thermoneutral and heat stress conditions.[1]

Treatment	Temp.[2]	Weight gain (g)	Feed intake (g)	Feed conversion ratio
Main Effect Means				
Diet				
1		2095.80[b]	4011.82[a]	1.86[a]
2		1856.43[c]	3316.82[c]	1.79[b]
3		2126.66[b]	3646.79[b]	1.72[c]
4		2106.02[b]	3565.38[b]	1.70[cd]
5		2291.19[a]	3687.71[b]	1.62[e]
6		2260.31[a]	3698.16[b]	1.65[de]
Temp.				
TN		2395.72[a]	4120.91[a]	1.71
HS		1849.75[b]	3187.99[b]	1.73
Source of Variation		----------Probabilities----------		
Diet		<0.0001	0.0003	<0.0001
Temp.		<0.0001	<0.0001	0.3905
Diet x Temp		0.2274	0.3214	0.2823
Pooled SEM		14.10	22.75	0.01

[1] Values are least square treatment means
[2] TN = thermoneutral environment (23.9°C), HS = heat stress environment (23.9-35°C)
[a, b, c, d, e] Main effect means within columns with different superscripts differ significantly (P<0.05)

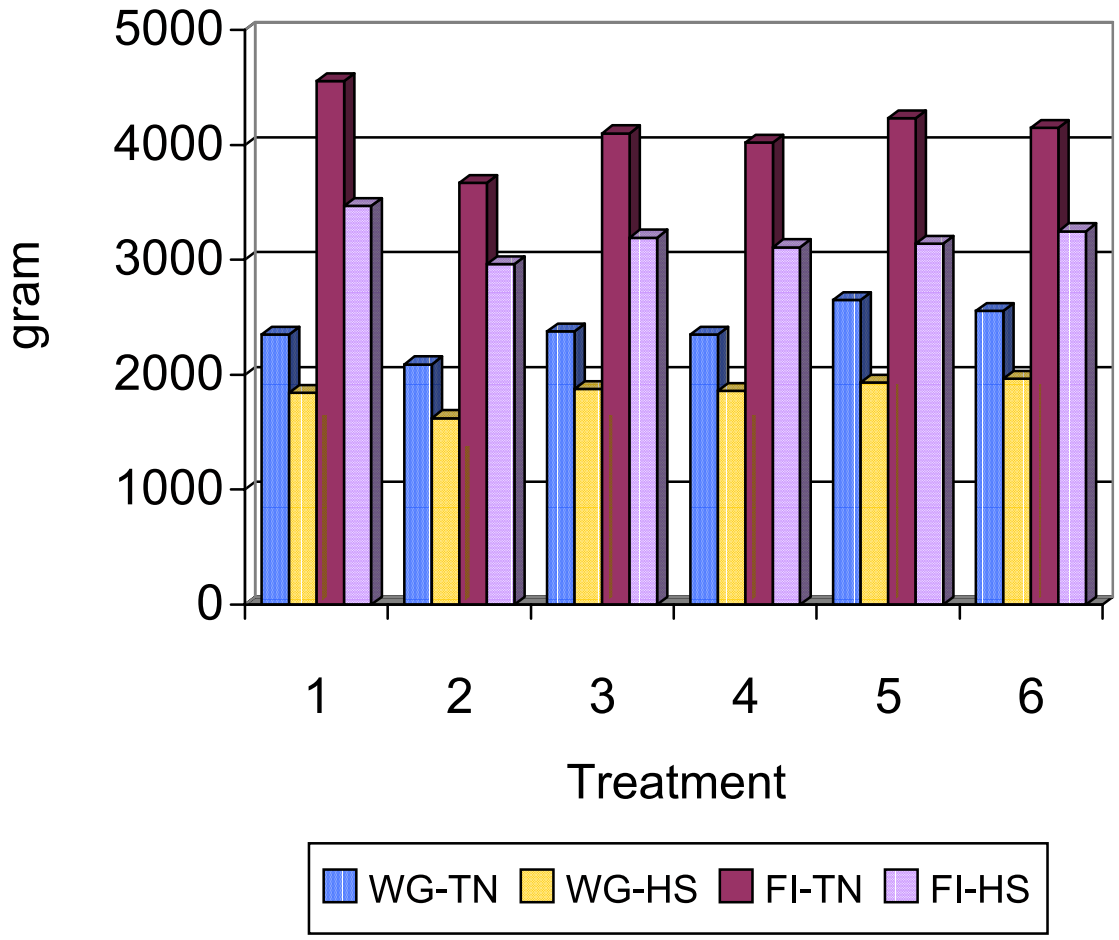


Figure 1. Weight gain (WG) and feed intake (FI) of 7-week-old broilers[1] fed different experimental diets[2] under thermoneutral (TN) and heat stress (HS) conditions.[3]

[1] Values are least square treatment means

[2] Treatment 1 = basal diet: corn-soybean diet
Treatment 2 = basal diet: 25% corn starch
Treatment 3 = diet with 15% corn starch, 10% defatted rice bran
Treatment 4 = diet with 15% corn starch, 10% defatted rice bran + phytase and xylanase
Treatment 5 = diet with 25% defatted rice bran
Treatment 6 = diet with 25% defatted rice bran + phytase and xylanase

[3] TN = thermoneutral environment (23.9^{o}C), HS = heat stress environment (23.9-35^{o}C)

(P<0.01). Birds fed 25% corn starch diet had the lowest body weight, weight gain, and feed intake. Birds fed 25% defatted rice bran diets had the highest body weight and weight gain. Feed intake of birds fed corn-soybean diet was highest. Different levels of defatted rice bran did not affect feed intake, but their influences were observed in feed conversion ratio. Defatted rice bran diets caused better feed conversion ratio with the highest level of defatted rice bran showing the best results. Broilers fed the corn-soybean diet had the worst feed conversion ratio. Adding both phytase and xylanase in the diets did not show an impact on any criteria examined, while the different temperatures did. Heat stress caused an adverse effect by significantly lowering weight gain, and feed intake of broilers during 3-7 week of age by approximately 30.3, and 27.8%, respectively (P<0.01). Feed conversion ratio was significantly increased by 4.3% because of heat stress condition (P<0.01).

Water Intake and Digesta Retention Time

Daily water intake and water:feed consumption ratio of broilers were affected (P<0.01) by both dietary and temperature treatments (Table 8). Birds fed 25% corn starch diet consumed significantly less water per day, however water:feed consumption ratio of birds fed both control diets was not significantly different (Figure 2). High level of defatted rice bran in diet caused the birds to consume significantly more water and resulted in a higher water:feed consumption ratio. Enzymes supplementation did not affect water intake of birds. Birds in the heat stress environment consumed significantly more water and had higher water:feed consumption ratio than birds raised in the thermoneutral environment, approximately 18.3% and 60.7%, respectively.

Table 8. Main effect means of daily water intake and water:feed consumption ratio of broilers during 3-7 week of age, and digesta retention time of 5-week-old broilers fed different experimental diets under thermoneutral and heat stress conditions.[1]

	Daily water intake (ml)	Water:feed consumption ratio	Digesta retention time (minute)
Main Effect Means			
Diet			
1	179.31[b]	1.54[c]	172.45
2	150.29[c]	1.72[c]	184.72
3	189.39[b]	1.94[b]	181.30
4	202.11[ab]	2.12[ab]	189.70
5	222.06[a]	2.19[a]	174.77
6	220.53[a]	2.23[a]	165.62
Temp.[2]			
TN	177.66[b]	1.50[b]	169.90[b]
HS	210.24[a]	2.41[a]	186.29[a]
Source of Variation	----------Probabilities----------		
Diet	0.0014	<0.0001	0.2849
Temp.	0.0012	<0.0001	0.0193
Diet x Temp	0.6672	0.3023	0.4698
Pooled SEM	2.98	0.03	2.58

[1] Values are least square treatment means
[2] TN = thermoneutral environment (23.9°C), HS = heat stress environment (23.9-35°C)
[a, b, c] Main effect means within columns with different superscripts differ significantly (P<0.05)

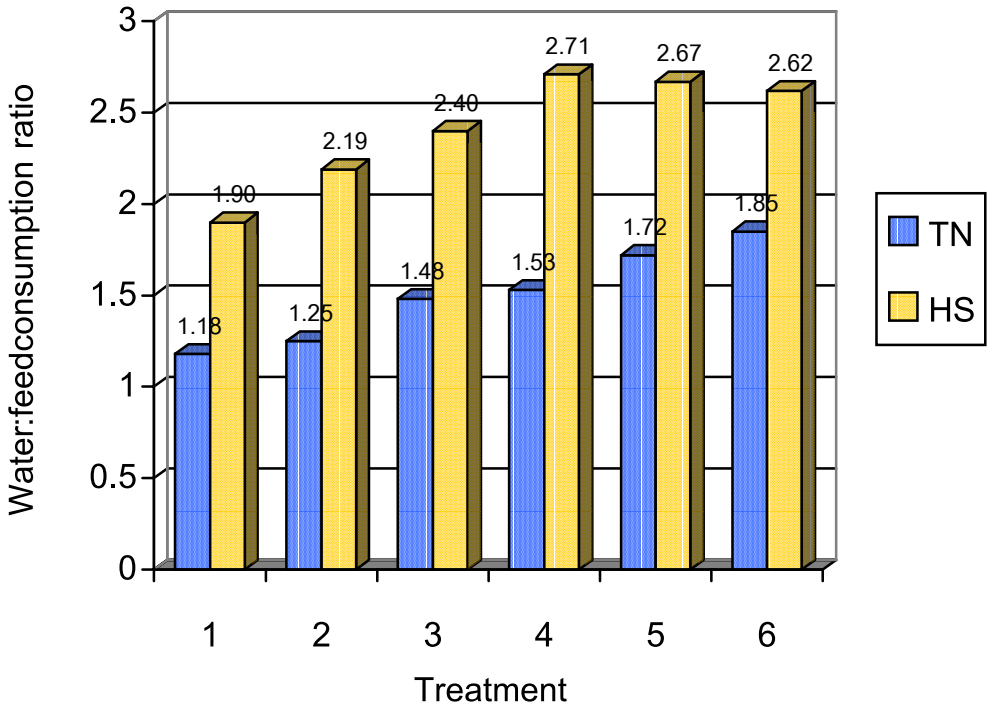


Figure 2. Water:feed consumption ratio of broilers[1] during 3-7 week of age fed different experimental diets[2] under thermoneutral (TN) and heat stress (HS) conditions.[3]

[1] Values are least square treatment means

[2] Treatment 1 = basal diet: corn-soybean diet
Treatment 2 = basal diet: 25% corn starch
Treatment 3 = diet with 15% corn starch, 10% defatted rice bran
Treatment 4 = diet with 15% corn starch, 10% defatted rice bran + phytase and xylanase
Treatment 5 = diet with 25% defatted rice bran
Treatment 6 = diet with 25% defatted rice bran + phytase and xylanase

[3] TN = thermoneutral environment (23.9^{o}C), HS = heat stress environment (23.9-35^{o}C)

Dietary treatments did not have an impact on the digesta retention time of 5-week-old broilers (Table 8, Figure 3). Heat stress broilers had significantly longer digesta retention time by approximately 9.6% (P=0.02).

Carcass Quality

Carcass percentage of 7-week-old birds was not affected by dietary treatments (Table 9), while abdominal fat was significantly increased in broilers fed diets containing high level of defatted rice bran (P<0.01). Inclusion of enzymes did not affect percent abdominal fat, however, heat stress caused a 2% increase (P<0.01) in percent carcass and lowerd abdominal fat percentage by 25.2% (P<0.01).

Differences in market portions of bird are shown in Table 10. Dietary treatments significantly influenced (P=0.05) the percentage of wings, while no differences were found in other market portions of birds. The corn starch diet significantly increased percentage of wings compared with diets containing high level of defatted rice bran. Enzyme inclusion did not influence percentage of wings. Heat stress was found to significantly decrease percentage of breast by approximately 6.3% (P<0.01), whereas percentages of legs and wings were increased by 6.6 and 4.8% respectively (P<0.01). Temperature treatments did not show any effect on percentage of thighs.

Gross Anatomical Observations of Intestine

Total intestinal weight as percentage of live weight of broilers was not influenced by treatment (Table 9), nor were the weight percentages of different parts of intestine

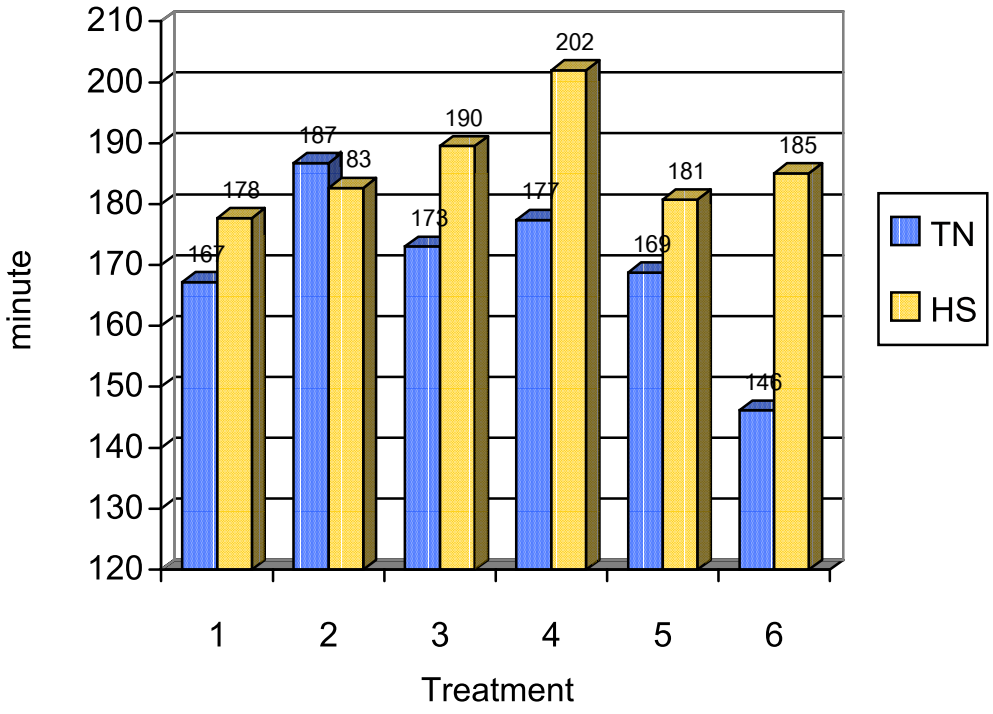


Figure 3. Digesta retention time of 5-week-old broilers[1] fed different experimental diets[2] under thermoneutral (TN) and heat stress (HS) conditions.[3]

[1] Values are least square treatment means

[2] Treatment 1 = basal diet: corn-soybean diet
Treatment 2 = basal diet: 25% corn starch
Treatment 3 = diet with 15% corn starch, 10% defatted rice bran
Treatment 4 = diet with 15% corn starch, 10% defatted rice bran + phytase and xylanase
Treatment 5 = diet with 25% defatted rice bran
Treatment 6 = diet with 25% defatted rice bran + phytase and xylanase

[3] TN = thermoneutral environment (23.9^{o}C), HS = heat stress environment (23.9-35^{o}C)

Table 9. Carcass, abdominal fat, and intestinal weight as percentages of live weight of 7-week-old broilers fed different experimental diets under thermoneutral and heat stress conditions.[1]

Treatment	Temp.[2]	Carcass (%)	Abdominal fat (%)	Intestine (%)
1	TN	69.38	2.29	1.34
	HS	70.30	1.93	1.31
2	TN	67.37	2.46	1.35
	HS	69.74	1.60	1.43
3	TN	69.18	2.71	1.29
	HS	68.96	1.96	1.22
4	TN	68.45	2.52	1.48
	HS	68.73	2.22	1.38
5	TN	66.96	4.25	1.36
	HS	69.51	3.03	1.21
6	TN	67.33	4.10	1.43
	HS	69.47	2.98	1.37
Pooled SEM		0.15	0.06	0.02
Source of Variation		----------Probabilities----------		
Diet		0.2486	0.0002	0.1489
Temp.		0.0052	0.0006	0.2145
Diet x Temp		0.2573	0.5118	0.6417
Main Effect Means				
Diet				
1		69.84	2.11[b]	1.32
2		68.55	2.03[b]	1.39
3		69.07	2.34[b]	1.26
4		68.59	2.37[b]	1.43
5		68.24	3.64[a]	1.29
6		68.40	3.54[a]	1.40
Temp.				
TN		68.11[b]	3.06[a]	1.37
HS		69.45[a]	2.29[b]	1.32

[1] Values are least square treatment means
[2] TN = thermoneutral environment (23.9°C), HS = heat stress environment (23.9-35°C)
[a, b] Main effect means within columns with different superscripts differ significantly ($P<0.05$)

Table 10. Breast, legs, thighs, and wings weight as percentages of carcass weight of 7-week-old broilers fed different experimental diets under thermoneutral and heat stress conditions.[1]

Treatment	Temp.[2]	Breast (%)	Legs (%)	Thighs (%)	Wings (%)
1	TN	33.61	13.71	17.74	12.38
	HS	30.83	14.91	17.65	12.90
2	TN	32.88	14.76	17.35	12.53
	HS	30.49	15.45	16.56	13.62
3	TN	32.41	14.51	17.32	12.18
	HS	30.94	14.95	17.93	13.16
4	TN	31.92	14.49	17.03	12.65
	HS	31.47	15.34	16.99	12.64
5	TN	32.47	13.75	17.58	11.90
	HS	30.81	14.99	18.43	12.43
6	TN	33.08	13.91	17.37	11.92
	HS	29.56	15.12	18.97	12.33
Pooled SEM		0.17	0.09	0.11	0.07
Source of Variation		----------Probabilities----------			
Diet		0.8018	0.1906	0.0529	0.0486
Temp.		0.0001	0.0005	0.1508	0.0040
Diet x Temp		0.2451	0.7780	0.1293	0.4462
Main Effect Means					
Diet					
1		32.22	14.31	17.69	12.64[ab]
2		31.69	15.11	16.96	13.08[a]
3		31.67	14.73	17.62	12.67[ab]
4		31.69	14.92	17.01	12.64[ab]
5		31.64	14.37	18.01	12.16[b]
6		31.32	14.51	18.17	12.12[b]
Temp.					
TN		32.73[a]	14.19[b]	17.40	12.26[b]
HS		30.68[b]	15.13[a]	17.76	12.85[a]

[1] Values are least square treatment means
[2] TN = thermoneutral environment (23.9°C), HS = heat stress environment (23.9-35°C)
[a, b] Main effect means within columns with different superscripts differ significantly ($P<0.05$)

different among dietary treatments (Table 11). Duodenum and jejunum weight percentages were influenced by environmental temperature ($P<0.01$). Heat stress increased duodenum weight and lowered jejunum weight percentages approximately 7.8 and 7.4%, respectively.

Intestinal Villi Height and Crypt Depth

There were no treatment differences found in villi height, crypt depth, or villi height:crypt depth ratio of both duodenum (Table 12) and jejunum (Table 13).

Tibia Mineral Content

Tibia dry matters of both 3-week-old and 7-week-old broilers were not affected by treatment (Table 14 and 16 respectively). Tibia ash and zinc content of 3-week-old chicks were not affected by dietary treatments (Table 14 and 15, respectively).

Percent tibia calcium of 3-week-old chicks was highest in birds fed corn-soybean diet ($P<0.01$), but it was not different from birds fed 25% defatted rice bran diets (Table 14). Including defatted rice bran 10% in the diet significantly decreased tibia calcium. Inclusion of enzymes increased percent tibia calcium in chicks fed defatted rice bran diets, but the differences were not significant. Percent tibia phosphorus of 3-week-old chicks was significantly higher in chicks fed 25% defatted rice bran diet supplemented with enzymes ($P<0.01$) compared to chicks fed other diets (Table 14). Because of high calcium content in the tibia of birds fed the corn-soybean diet, calcium:phosphorus ratio in tibia was significantly higher in these birds ($P<0.01$). The ratio of calcium:phosphorus

Table 11. Duodenum, jejunum, ileum, ceca, and colon weight as percentages of intestinal weight of 7-week-old broilers fed different experimental diets under thermoneutral and heat stress conditions.[1]

Treatment	Temp.[2]	Duodenum (%)	Jejunum (%)	Ileum (%)	Ceca (%)	Colon (%)
1	TN	15.39	35.15	27.75	14.29	6.11
	HS	16.96	31.59	28.80	15.22	8.91
2	TN	17.41	33.19	28.39	13.48	7.52
	HS	18.71	32.03	27.12	14.61	7.53
3	TN	16.93	36.62	26.92	13.34	6.82
	HS	17.31	33.63	26.56	14.37	8.13
4	TN	15.71	37.02	27.27	13.52	6.48
	HS	16.92	32.56	28.16	14.14	8.23
5	TN	16.43	34.26	27.00	14.14	8.12
	HS	18.77	33.02	26.36	14.79	7.06
6	TN	16.83	33.20	28.05	14.50	7.42
	HS	17.73	31.22	29.14	14.30	7.61
Pooled SEM		0.17	0.26	0.26	0.21	0.22
Source of Variation		----------Probabilities----------				
Diet		0.0963	0.1542	0.3744	0.9395	0.9998
Temp.		0.0065	0.0026	0.8301	0.2895	0.1157
Diet x Temp		0.7777	0.6374	0.7454	0.9899	0.3196
Main Effect Means						
Diet						
1		16.17	33.37	28.27	14.75	7.51
2		18.06	32.61	27.76	14.04	7.53
3		17.12	35.13	26.74	13.86	7.48
4		16.32	34.79	27.71	13.83	7.35
5		17.60	33.64	26.68	14.46	7.59
6		17.28	32.21	28.60	14.40	7.51
Temp.						
TN		16.45[b]	34.91[a]	27.56	13.88	7.08
HS		17.73[a]	32.34[b]	27.69	14.57	7.91

[1] Values are least square treatment means
[2] TN = thermoneutral environment (23.9°C), HS = heat stress environment (23.9-35°C)
[a, b] Main effect means within columns with different superscripts differ significantly (P<0.05)

Table 12. Duodenal villi height, crypt depth, and villi height:crypt depth ratio of 7-week-old broilers fed different experimental diets under thermoneutral and heat stress conditions.[1]

Treatment	Temp.[2]	Villi height (mm)	Crypt depth (mm)	Villi height: crypt depth ratio
1	TN	2.43	0.40	6.27
	HS	2.78	0.43	6.60
2	TN	2.57	0.39	6.76
	HS	2.66	0.38	7.12
3	TN	2.59	0.40	6.09
	HS	2.48	0.39	6.42
4	TN	2.54	0.43	6.40
	HS	2.71	0.41	6.82
5	TN	2.89	0.42	6.54
	HS	2.95	0.41	6.30
6	TN	2.74	0.44	6.52
	HS	2.24	0.40	5.79
Pooled SEM		0.04	0.007	0.15
Source of Variation		----------Probabilities----------		
Diet		0.2716	0.7280	0.8007
Temp.		0.9052	0.5304	0.8203
Diet x Temp		0.2129	0.8379	0.8960
Main Effect Means				
Diet				
1		2.61	0.42	6.43
2		2.62	0.38	6.94
3		2.53	0.40	6.25
4		2.62	0.42	6.61
5		2.92	0.41	6.42
6		2.49	0.42	6.15
Temp.				
TN		2.63	0.41	6.43
HS		2.64	0.40	6.51

[1] Values are least square treatment means
[2] TN = thermoneutral environment (23.9^{o}C), HS = heat stress environment (23.9-35^{o}C)

Table 13. Jejunal villi height, crypt depth, and villi height:crypt depth ratio of 7-week-old broilers fed different experimental diets under thermoneutral and heat stress conditions.[1]

Treatment	Temp.[2]	Villi height (mm)	Crypt depth (mm)	Villi height: crypt depth ratio
1	TN	2.20	0.33	6.37
	HS	2.43	0.36	6.92
2	TN	2.49	0.39	6.57
	HS	2.64	0.35	7.55
3	TN	2.59	0.37	7.36
	HS	2.50	0.37	6.95
4	TN	2.47	0.34	7.49
	HS	2.11	0.34	6.45
5	TN	2.56	0.33	7.93
	HS	2.23	0.37	6.06
6	TN	2.49	0.35	6.52
	HS	2.09	0.33	6.48
Pooled SEM		0.04	0.004	0.12
Source of Variation		----------Probabilities----------		
Diet		0.4550	0.3053	0.7557
Temp.		0.2225	0.8310	0.3094
Diet x Temp		0.3933	0.2953	0.1164
Main Effect Means				
Diet				
1		2.31	0.34	6.64
2		2.57	0.37	7.06
3		2.55	0.37	7.16
4		2.29	0.34	6.97
5		2.40	0.35	6.99
6		2.29	0.34	6.50
Temp.				
TN		2.47	0.35	7.04
HS		2.33	0.35	6.74

[1] Values are least square treatment means
[2] TN = thermoneutral environment (23.9°C), HS = heat stress environment (23.9-35°C)

Table 14. Dry matter, ash, calcium and phosphorus content, and calcium:phosphorus ratio in tibia of 3-week-old broilers fed different experimental diets.[1]

Treatment	Dry matter (%)	Ash (%)	Calcium (%)	Phosphorus (%)	Calcium:phosphorus ratio
1	86.91	62.44	35.12[a]	21.28[b]	1.65[a]
2	88.41	62.10	33.70[bc]	21.26[b]	1.59[b]
3	85.46	62.75	32.63[d]	21.44[b]	1.52[c]
4	86.12	62.07	33.48[cd]	21.35[b]	1.57[b]
5	88.31	61.88	34.37[ab]	21.46[b]	1.60[b]
6	88.03	61.20	34.89[a]	21.98[a]	1.59[b]
Pooled SEM	0.38	0.14	0.12	0.06	0.006
Probabilities	0.1313	0.0665	<0.0001	0.0063	<0.0001

[1] Values are least square treatment means
[a, b, c, d] Means within columns with different superscripts differ significantly (P<0.05)

Table 15. Zinc and manganese content in tibia of 3-week-old broilers fed different experimental diets.[1]

Treatment	Zinc (ug/g)	Manganese (ug/g)
1	426.27	8.41[c]
2	424.51	8.95[bc]
3	435.52	8.76[bc]
4	416.78	9.33[ab]
5	411.57	8.69[c]
6	430.30	9.62[a]
Pooled SEM	2.34	0.09
Probabilities	0.0606	0.0026

[1] Values are least square treatment means
[a, b, c] Means within columns with different superscripts differ significantly (P<0.05)

Table 16. Dry matter, ash, calcium and phosphorus content, and calcium:phosphorus ratio in tibia of 7-week-old broilers fed different experimental diets under thermoneutral and heat stress conditions.[1]

Treatment	Temp.[2]	Dry matter (%)	Ash (%)	Calcium (%)	Phosphorus (%)	Calcium:phosphorus ratio
1	TN	88.30	58.42	33.99	18.59	1.87
	HS	86.75	59.44	33.74	19.23	1.75
2	TN	84.82	59.35	32.03	17.66	1.82
	HS	86.35	59.57	33.12	18.80	1.76
3	TN	87.93	58.52	32.85	18.32	1.80
	HS	87.39	59.62	32.55	18.96	1.72
4	TN	88.21	58.64	33.57	19.08	1.76
	HS	87.56	59.57	33.37	19.63	1.70
5	TN	88.27	58.17	33.21	19.21	1.73
	HS	89.28	59.57	32.55	19.79	1.65
6	TN	88.22	57.83	33.36	19.20	1.74
	HS	88.03	60.04	31.82	19.18	1.66
Pooled SEM		0.18	0.13	0.19	0.04	0.01
Source of Variation		----------Probabilities----------				
Diet		0.0859	0.8411	0.6805	<0.0001	0.3130
Temp.		0.9081	0.0018	0.5850	<0.0001	0.0453
Diet x Temp		0.6132	0.4947	0.8376	0.0671	0.9953
Main Effect Means						
Diet						
1		87.52	58.93	33.86	18.91[bc]	1.81
2		85.58	59.46	32.58	18.23[d]	1.79
3		87.66	59.07	32.70	18.64[c]	1.76
4		87.89	59.10	33.47	19.35[a]	1.73
5		88.78	58.87	32.88	19.50[a]	1.69
6		88.12	58.93	32.59	19.19[ab]	1.70
Temp.						
TN		87.62	58.49[b]	33.17	18.68[b]	1.79[a]
HS		87.56	59.63[a]	32.86	19.26[a]	1.71[b]

[1] Values are least square treatment means
[2] TN = thermoneutral environment (23.9°C), HS = heat stress environment (23.9-35°C)
[a, b, c, d] Main effect means within columns with different superscripts differ significantly (P<0.05)

was lowest in birds fed 10% defatted rice bran diet without enzymes supplementation.

Enzyme supplementation also significantly improved tibia manganese content in defatted rice bran diets ($P<0.01$), but was significant only in birds fed 25% defatted rice bran diet (Table 15). However, enzyme supplementation in 10% defatted rice bran diet resulted in significantly higher manganese content compared to birds fed corn-soybean diet and 25% defatted rice bran diet without supplementation of enzymes.

Dietary treatments did not affect percent tibia ash of 7-week-old-broilers (Table 16), however, heat stress significantly increased ash percentage by 1.9% ($P<0.01$). No significant treatment effects were found in percent tibia calcium and zinc content of 7-week-old broilers, while both dietary and temperature treatments significantly affected percent phosphorus and manganese content (Table 16 and 17). Percent phosphorus was lowest ($P<0.01$) in tibia of birds fed 25% corn starch diet. Defatted rice bran diets increased tibia phosphorus content. Enzyme supplementation significantly improved percent phosphorus in 10% defatted rice bran diet by 3.8%, but did not affect the 25% defatted rice bran diet. However, from calculation of the calcium:phosphorus ratio, no differences were found between dietary treatments. Birds raised under heat stress condition had significantly higher percentage of phosphorus, approximately 3.1% ($P<0.01$). Therefore, the ratio of calcium:phosphorus in tibia was significantly lower under heat stress condition ($P=0.05$).

Manganese content in tibia was lowest in the 25% defatted rice bran diet supplemented with enzymes ($P<0.01$), but did not differ from the 25% defatted rice bran

Table 17. Zinc and manganese content in tibia of 7-week-old broilers fed different experimental diets under thermoneutral and heat stress conditions.[1]

Treatment	Temp.[2]	Zinc (ug/g)	Manganese (ug/g)
1	TN	339.89	10.43
	HS	337.37	9.51
2	TN	320.33	10.90
	HS	331.24	10.54
3	TN	328.49	11.80
	HS	325.54	9.66
4	TN	335.69	11.12
	HS	333.75	10.04
5	TN	332.05	10.78
	HS	325.48	9.22
6	TN	333.60	9.92
	HS	318.21	8.59
Pooled SEM		1.93	0.10
Source of Variation		----------Probabilities----------	
Diet		0.6805	0.0086
Temp.		0.5851	<0.0001
Diet x Temp		0.8376	0.2590
Main Effect Means			
Diet			
1		338.63	9.97[ab]
2		325.79	10.72[a]
3		327.02	10.73[a]
4		334.72	10.58[a]
5		328.77	10.00[ab]
6		325.90	9.26[b]
Temp.			
TN		331.68	10.82[a]
HS		328.60	9.59[b]

[1] Values are least square treatment means
[2] TN = thermoneutral environment (23.9°C), HS = heat stress environment (23.9-35°C)
[a, b, c, d] Main effect means within columns with different superscripts differ significantly (P<0.05)

diet without enzymes supplementation or the corn-soybean diet. Enzyme inclusion did not affect the manganese content of the 10% defatted rice bran diet. Heat stress decreased manganese content in tibia by 11.4% ($P<0.01$).

Calcium and Phosphorus Retention

Amount of excreta (dry matter basis) as percentage of feed consumed by 5-week-old broilers is shown in figure 4. Birds fed 25% defatted rice bran diets had higher amount of excreta and enzyme supplementation lowered that amount significantly. The results from analysis of calcium and phosphorus in excreta of 5-week-old broilers are presented in Table 18 and Figure 5. Significant differences between dietary treatments were observed in percent calcium, percent phosphorus, and their retentions (Figure 6). No differences were observed between temperature treatments in all criteria examined, except percent calcium that was decreased by 8.0% under heat stress condition ($P=0.04$).

Calcium level in the excreta was highest in the corn-soybean diet and lowest in diets containing 25% defatted rice bran ($P<0.01$). Enzyme supplementation had no effect on excreta calcium of birds on the 25% defatted rice bran diet. The diet containing 10% defatted rice bran had significantly lower calcium than control diets. Adding enzyme to that diet increased excreta calcium to the same level as the 25% corn starch diet, but it was not significant different from the 10% defatted rice bran diet without enzyme supplementation. From the calculation of calcium retention, birds fed corn-soybean diet had the highest retention but this was not significantly different from birds fed 25% corn starch diet or 10% defatted rice bran diet without enzymes supplementation ($P<0.01$).

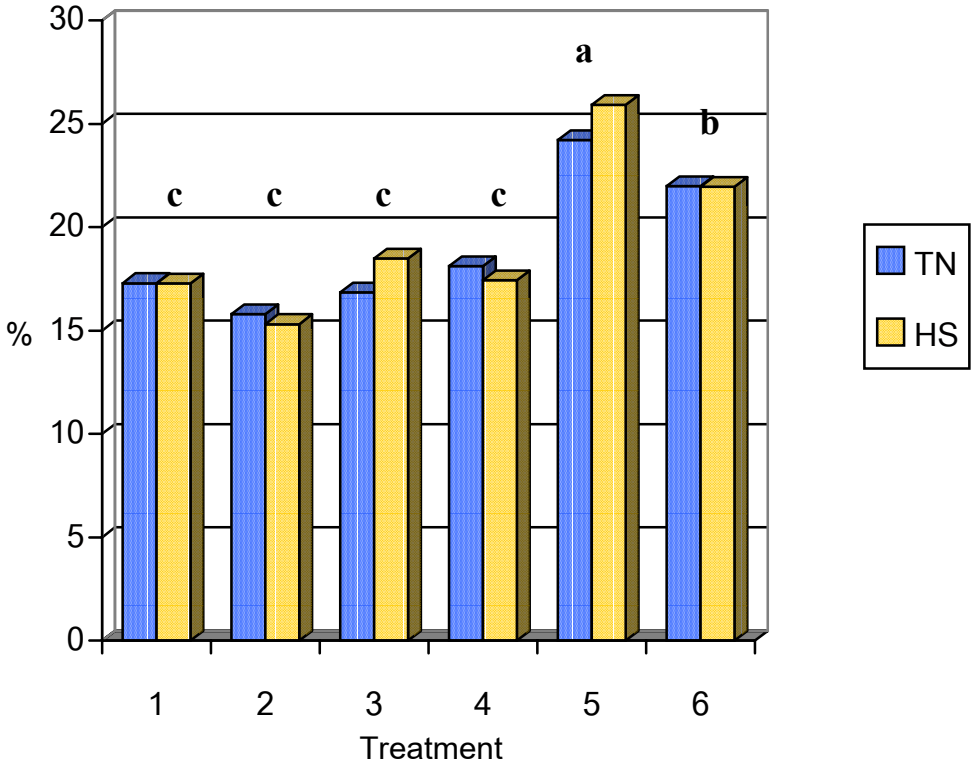


Figure 4. Amount of excreta (dry matter basis) as percentage of feed consumed by 5-week-old broilers[1] fed different experimental diets[2] under thermoneutral (TN) and heat stress (HS) conditions.[3]

[1] Values are least square treatment means, pooled SEM = 0.22

[2] Treatment 1 = basal diet: corn-soybean diet
Treatment 2 = basal diet: 25% corn starch
Treatment 3 = diet with 15% corn starch, 10% defatted rice bran
Treatment 4 = diet with 15% corn starch, 10% defatted rice bran + phytase and xylanase
Treatment 5 = diet with 25% defatted rice bran
Treatment 6 = diet with 25% defatted rice bran + phytase and xylanase

[3] TN = thermoneutral environment (23.9°C), HS = heat stress environment (23.9-35°C)

[a, b, c] Main effect means of dietary treatments with different superscripts differ significantly ($P<0.0001$)

Table 18. Percent calcium and phosphorus in excreta, and their retention by 5-week-old broilers fed different experimental diets under thermoneutral and heat stress conditions.[1]

Treatment	Temp.[2]	Calcium (%)	Calcium retention (%)	Phosphorus (%)	Phosphorus retention (%)
Main Effect Means					
Diet					
1		3.08[a]	56.54[a]	2.15[c]	58.29[a]
2		2.77[ab]	49.60[ab]	2.17[c]	53.22[a]
3		2.43[c]	50.95[ab]	2.68[b]	45.33[b]
4		2.70[bc]	41.25[c]	2.68[b]	41.56[b]
5		2.08[d]	38.95[c]	3.21[a]	13.19[d]
6		2.06[d]	45.16[bc]	2.89[b]	25.41[c]
Temp.					
TN		2.63[a]	45.57	2.64	38.77
HS		2.42[b]	48.58	2.62	40.24
Source of Variation		----------Probabilities----------			
Diet		0.0002	0.0058	<0.0001	<0.0001
Temp.		0.0363	0.1843	0.8110	0.5136
Diet x Temp		0.6031	0.9715	0.0549	0.6212
Pooled SEM		0.04	0.92	0.03	0.86

[1] Values are least square treatment means
[2] TN = thermoneutral environment (23.9°C), HS = heat stress environment (23.9-35°C)
[a, b] Main effect means within columns with different superscripts differ significantly (P<0.05)

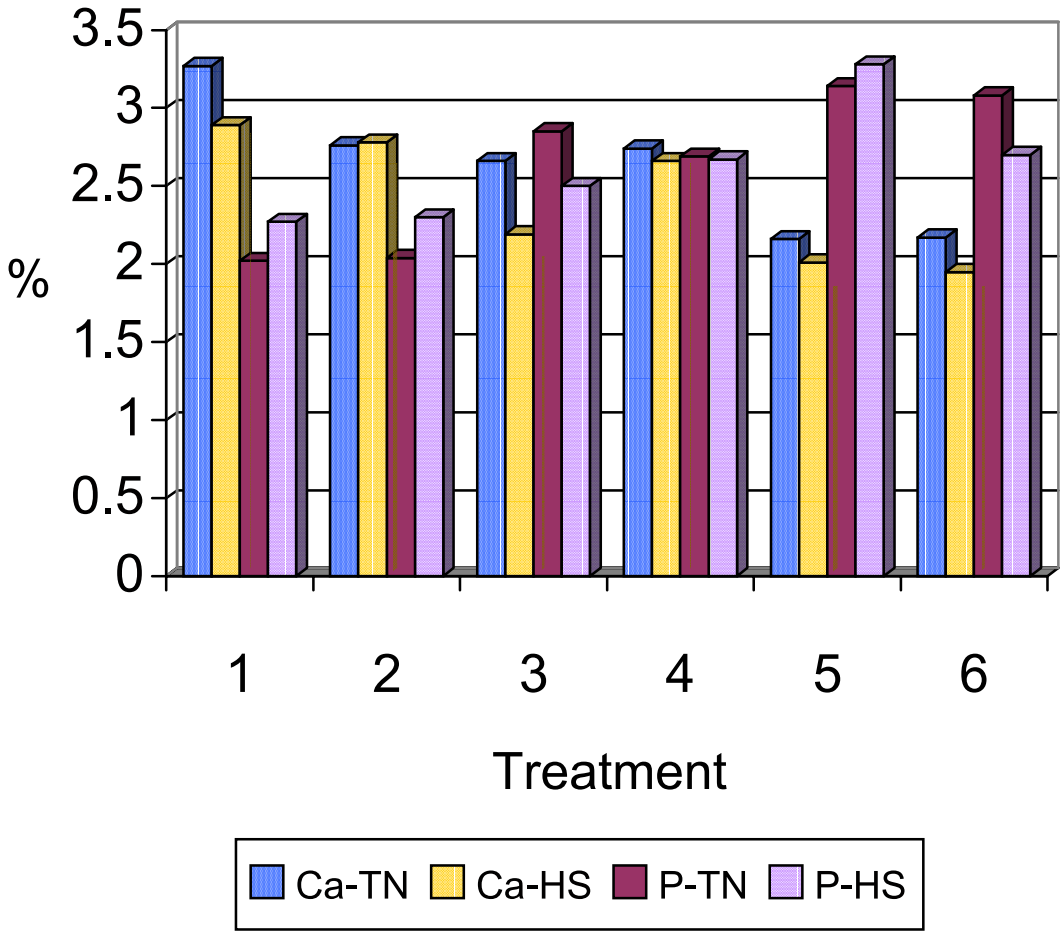


Figure 5. Percent calcium (Ca) and phosphorus (P) in excreta by 5-week-old broilers[1] fed different experimental diets[2] under thermoneutral (TN) and heat stress (HS) conditions.[3]

[1] Values are least square treatment means

[2] Treatment 1 = basal diet: corn-soybean diet
Treatment 2 = basal diet: 25% corn starch
Treatment 3 = diet with 15% corn starch, 10% defatted rice bran
Treatment 4 = diet with 15% corn starch, 10% defatted rice bran + phytase and xylanase
Treatment 5 = diet with 25% defatted rice bran
Treatment 6 = diet with 25% defatted rice bran + phytase and xylanase

[3] TN = thermoneutral environment (23.9^{o}C), HS = heat stress environment (23.9-35^{o}C)

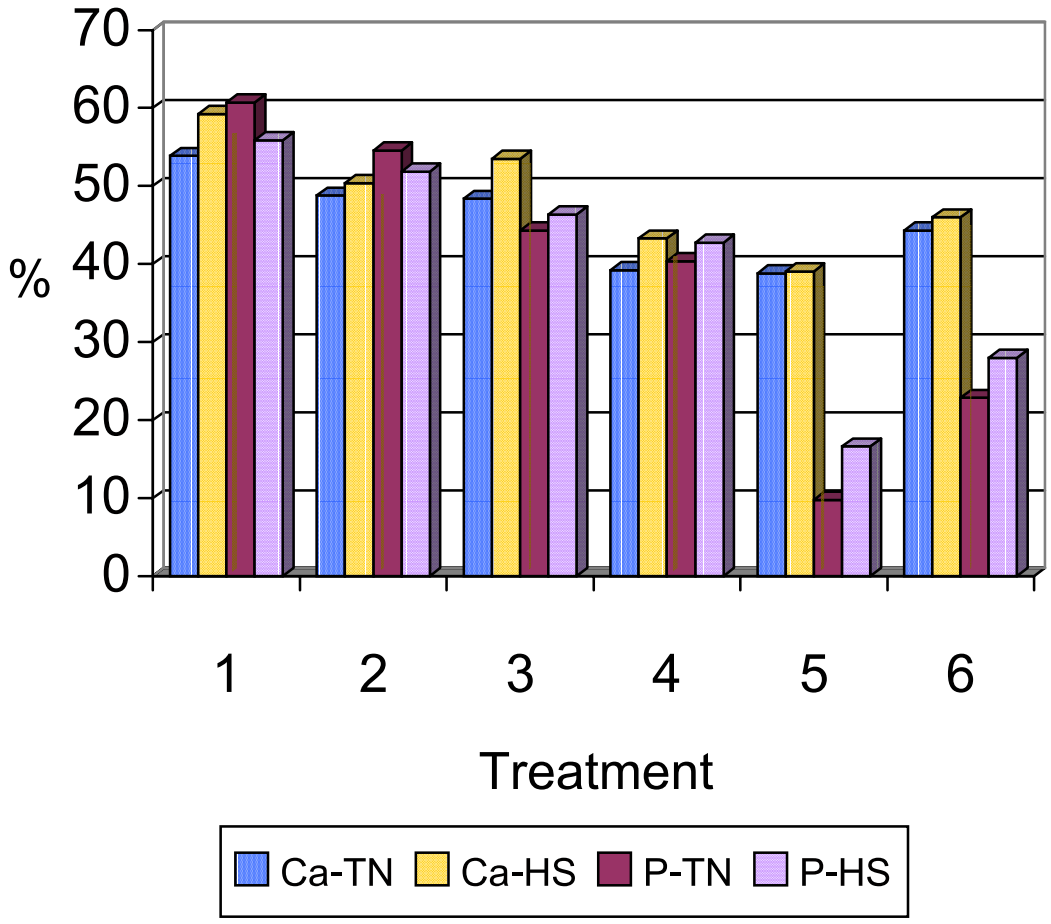


Figure 6. Percent calcium (Ca) and phosphorus (P) retention by 5-week-old broilers[1] fed different experimental diets[2] under thermoneutral (TN) and heat stress (HS) conditions.[3]

[1] Values are least square treatment means

[2] Treatment 1 = basal diet: corn-soybean diet
Treatment 2 = basal diet: 25% corn starch
Treatment 3 = diet with 15% corn starch, 10% defatted rice bran
Treatment 4 = diet with 15% corn starch, 10% defatted rice bran + phytase and xylanase
Treatment 5 = diet with 25% defatted rice bran
Treatment 6 = diet with 25% defatted rice bran + phytase and xylanase

[3] TN = thermoneutral environment (23.9^{o}C), HS = heat stress environment (23.9-35^{o}C)

Enzyme supplementation significantly decreased calcium retention of birds fed 10% defatted rice bran diet, but did not have an impact on birds fed 25% defatted rice bran diet.

The phosphorus content of the excreta was significantly higher in broilers fed defatted rice bran diets ($P<0.01$). Without enzymes supplementation, the excreta of birds on the 25% defatted rice bran diet had significantly higher phosphorus percentage. Enzyme supplementation had no effect on excreta phosphorus of birds on the 10% defatted rice bran diet. Because of lower phosphorus loss in the excreta, percent phosphorus retention was highest in control diets ($P<0.01$). Birds fed high level of defatted rice bran had significantly lower phosphorus retention. Including both phytase and xylanase in that diet improved phosphorus retention by 92.6%, however this level still significantly lower than in the other groups.

Apparent Metabolizable Energy

Apparent metabolizable energy of the diets of 5-week-old broilers is shown in Table 19 and Figure 7. Dietary treatments caused significant differences in apparent metabolizable energy ($P<0.01$). The birds fed 25% defatted rice bran diets had highest apparent metabolizable energy, while birds fed control diets had lowest values. Enzyme supplementation or different temperature treatments did not affect apparent metabolizable energy.

Table 19. Apparent metabolizable energy of different experimental diets of 5-week-old broilers under thermoneutral and heat stress conditions.[1]

Treatment	Apparent metabolizable energy (kcal/kg)
Main Effect Means	
Diet	
1	3398.51[d]
2	3390.57[d]
3	3519.25[bc]
4	3493.02[cd]
5	3633.65[ab]
6	3696.37[a]
Temp.	
TN	3541.98
HS	3501.80
Source of Variation	----------Probabilities----------
Diet	0.0004
Temp.	0.1974
Diet x Temp	0.9074
Pooled SEM	8.95

[1] Values are least square treatment means
[a, b, c, d] Main effect means with different superscripts differ significantly ($P<0.05$)

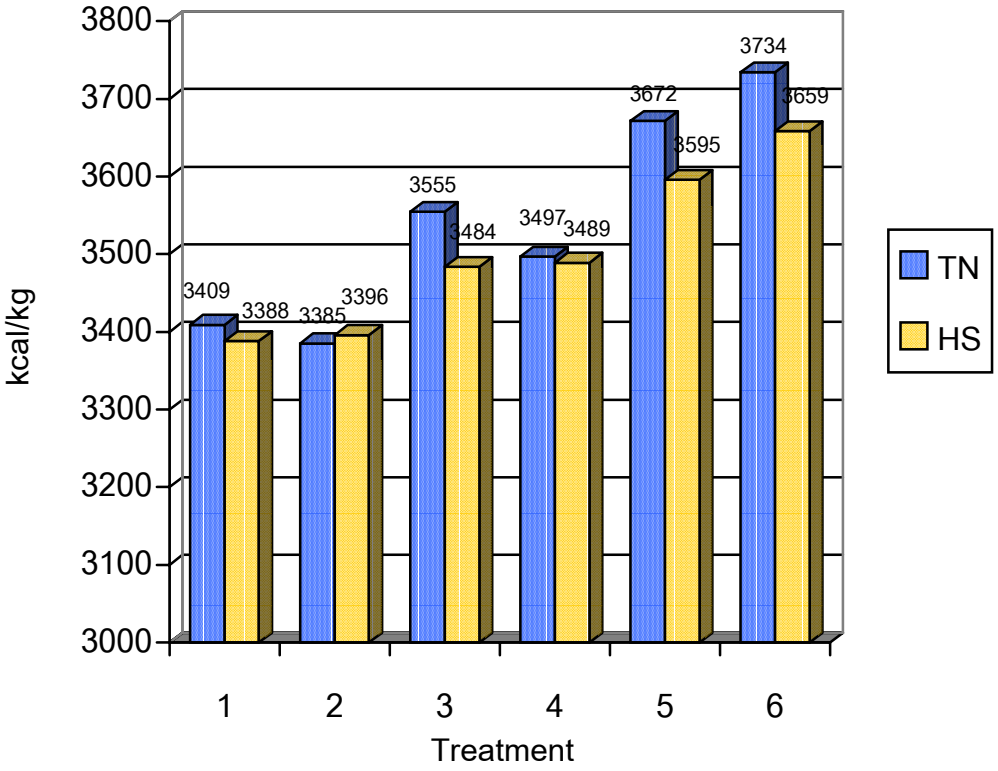


Figure 7. Apparent metabolizable energy[1] of different experimental diets[2] of 5-week-old broilers under thermoneutral (TN) and heat stress (HS) conditions.[3]

[1] Values are least square treatment means

[2] Treatment 1 = basal diet: corn-soybean diet
Treatment 2 = basal diet: 25% corn starch
Treatment 3 = diet with 15% corn starch, 10% defatted rice bran
Treatment 4 = diet with 15% corn starch, 10% defatted rice bran + phytase and xylanase
Treatment 5 = diet with 25% defatted rice bran
Treatment 6 = diet with 25% defatted rice bran + phytase and xylanase

[3] TN = thermoneutral environment (23.9^{o}C), HS = heat stress environment (23.9-35^{o}C)

Feed Cost per Weight Gain

Feed cost per pound of diet, calculated from the price of feed ingredients used in this experiment, is shown in Table 20. Feed cost per weight gain of birds during 0-3 week of age, as shown in Table 21, was lowest in birds fed 25% defatted rice bran diet without enzyme supplementation ($P<0.01$). Adding enzyme in 25% defatted rice bran diet increased feed cost per weight gain, however it did not differed from that of corn-soybean based diet or 10% defatted rice bran diet with enzyme supplementation. Without enzyme supplementation, 10% defatted rice bran diet had higher feed cost per weight gain than corn-soybean based diet, but the highest feed cost per weight gain was detected in chicks fed corn starch diet.

During the 3-7 and 0-7 week periods, bird fed corn starch diets had significantly highest feed cost per weight gain (Table 22), where 25% defatted rice bran diets had lowest values ($P<0.01$). Feed cost per weight gain of birds fed corn-soybean based diet during 3-7 week of age did not differed from that of birds fed 10% defatted rice bran diet with enzyme supplementation, while without enzyme addition in that diet significantly lower feed cost per weight gain.

During 0-7 week of age, feed cost per weight gain of birds fed 25% defatted rice bran diet without enzyme supplementation was the lowest. Birds fed corn-soybean based diet had higher feed cost per weight gain than birds fed 25% defatted rice bran diets, but did not differed from birds fed 10% defatted rice bran diets. Heat stress caused a

Table 20. Feed cost (dollar) per pound diet of different experimental diets.[1]

Diet	Treatment					
	1	2	3	4	5	6
Starter	0.15	0.17	0.16	0.16	0.14	0.14
Grower	0.14	0.16	0.15	0.15	0.12	0.13
Finisher	0.13	0.15	0.14	0.14	0.12	0.12

[1] Price of feed ingredients (dollar/pound): Corn, 0.102; Corn starch, 0.18; Defatted rice bran, 0.032; Soybean meal, 0.134; Corn gluten meal, 0.27; Fish meal, 0.35; Tallow, 0.21; Vitamin Mix, 0.80; Dicalcium phosphate, 0.19; Limestone, 0.05; Salt, 0.10; L-Lysine, 1.03; DL-Methionine, 3.60; Salinomycin, 0.89; Sand, 0.06; Phytase, 2.40; Xylanase, 2.10.

Table 21. Feed cost per weight gain of broilers fed different experimental diets during 0-3 week of age.[1]

Treatment	Feed cost per weight gain 0-3 wk (dollars per pound)
1	0.27^{c}
2	0.34^{a}
3	0.30^{b}
4	0.26^{c}
5	0.21^{d}
6	0.25^{c}
Pooled SEM	0.003
Probabilities	<0.0001

[1] Values are least square treatment means
[a, b, c, d] Means with different superscripts differ significantly ($P<0.05$)

Table 22. Feed cost per weight gain of broilers fed different experimental diets during 3-7 and 0-7 week of age under thermoneutral and heat stress conditions.[1]

Treatment	Temp.[2]	3-7 wk	0-7 wk
		----------(dollars per pound)----------	
1	TN	0.28	0.27
	HS	0.29	0.27
2	TN	0.29	0.30
	HS	0.31	0.31
3	TN	0.26	0.27
	HS	0.27	0.27
4	TN	0.28	0.27
	HS	0.28	0.27
5	TN	0.22	0.21
	HS	0.23	0.22
6	TN	0.22	0.22
	HS	0.22	0.23
Pooled SEM		0.001	0.001
Source of Variation		----------Probabilities----------	
Diet		<0.0001	<0.0001
Temp.		0.0167	0.0292
Diet x Temp		0.4357	0.2047
Main Effect Means			
Diet			
1		0.28[b]	0.27[b]
2		0.30[a]	0.30[a]
3		0.27[c]	0.27[b]
4		0.28[b]	0.27[b]
5		0.22[d]	0.21[d]
6		0.22[d]	0.22[c]
Temp.			
TN		0.26[b]	0.25[b]
HS		0.27[a]	0.26[a]

[1] Values are least square treatment means
[2] TN = thermoneutral environment (23.9°C), HS = heat stress environment (23.9-35°C)
[a, b, c, d] Main effect means within columns with different superscripts differ significantly (P<0.05)

significant increase in feed cost per weight gain of birds during 3-7 and 0-7 week of age by approximately 3.85% (P=0.02) and 4% (P=0.03), respectively.

Discussion

To improve nutritional value of defatted rice bran diets, phytase and xylanase were added in the diets to reduce the negative effects of phytate and non-starch polysaccharide. In a previous experiment, only one control diet with 25% corn starch was used. The results indicated that inclusion of high level of corn starch in powder form may reduce palatability of that diet. Therefore, the birds consumed less feed and had lower body weight and weight gain compared to birds fed the other diets. To prove that assumption, in the current experiment another corn-soybean meal diet without corn starch was used as the other control diet. The substitution of corn starch with 10 and 25% defatted rice bran was done in treatment 3-4 and 5-6, respectively. Feed intake of birds fed the corn starch diet was less than for birds fed the corn-soybean diet and this difference was significant in 7-week-old broilers (Table 6 and 7). Body weight and weight gain of birds fed the corn starch diets were significantly lower than for birds fed the corn-soybean meal diets in both 3- and 7-week-old (Table 5-7). The results from the present experiment indicated that using a high level of corn starch in diet in powder form was detrimental to growth performance of broilers.

Feed intake of 3-week-old chicks fed both control diets was significantly lower than that of chicks fed the defatted rice bran diets except for chicks fed the 10% defatted

rice bran diet with enzyme supplementation. However, chicks fed the 10% defatted rice bran diet supplemented with enzymes had significantly better feed conversion ratio than chicks fed the control diets and the 10% defatted rice bran diet without enzyme supplementation. Chicks fed the 25% defatted rice bran diet without enzyme supplementation had significantly highest body weight gain and lowest feed conversion ratio, but the difference in feed conversion ratio of chicks in this group was not different from chicks fed the 10% defatted rice bran diet with enzymes. These results demonstrate that supplementation of both phytase and xylanase in the 10% defatted rice bran diet improved growth performance of chicks during starter period.

In this experiment, chicks fed 25% defatted rice bran diets without enzyme supplementation had significantly higher weight and weight gain than chicks fed 25% defatted rice bran diet supplemented with enzyme. This may be due to the possibility that ratio of male to female chicks in treatment 5 (25% defatted rice bran diet without enzyme supplementation) was higher than the ratio in treatment 6 (25% defatted rice bran diet with enzyme supplementation). Since the mixed sex day-old chicks were used during 0-3 week period, it was possible that the higher number of male chicks in treatment 5 caused the average weight and weight gain to be higher than those of treatment 6. During 3-7 week of age, the difference between weight and weight gain of these two treatments were decreased because the ratio of male:female used in this period were equal.

At 7-weeks of age, birds fed the corn starch diet had the lowest feed intake, body weight, and weight gain, as a result of lower palatability of diet. Birds fed the corn-soybean diet had the highest feed intake and feed conversion ratio. No significant

differences in feed intake were found among birds fed the defatted rice bran diets. However, the high defatted rice bran diets significantly increased the body weight and weight gain, therefore the feed conversion ratio was improved. These results were similar to the previous experiment, which indicated a positive effect of high levels of fat in the diets. Because of low metabolizable energy of the defatted rice bran, tallow was added to adjust the overall metabolizable energy of the diets. In grower diets, tallow was added in dietary treatment 3, 4, 5, and 6 by 3.12, 2.94, 9.06, and 8.88%, respectively. The high amount of fat in 25% defatted rice bran diets probably resulted in significantly higher apparent metabolizable energy in 5-week old broilers (Table 19) due to the extra caloric effect. Both control diets had the significant lower apparent metabolizable energy.

The ME_n value of feed grade fat has been shown to underestimate the net energy or effective energy of fat in both layers and broilers (Coon, 2001a). The beneficial effect of high-fat diets that lead to more metabolizable energy than the predicted energy value on the calculation basis from summation of ingredients was demonstrated in many studies (Mateos and Sell, 1980; Nitsan et al., 1997; Adrizal and Yayota, 2002). The extra caloric effect is increased for 10-20% from fats for maintenance and production compared to carbohydrates (Coon, 2001a). Although young broiler chicks had low lipase activity with relatively low bile secretion during the first weeks of life (Green and Kellog, 1987), they could tolerate high levels of dietary fat when the increase in lipase activity was detected during the second week of age (Adrizal and Yayota, 2002). In the present experiment, the enzyme supplementation did not affect body weight, weight gain, or feed conversion

ratio of 7-week-old birds. Apparent metabolizable energy was not affected by the enzyme supplementation. Bolton (1955) stated that increased digestibility of pentosans occurred in mature birds. The adult birds may have less trouble with viscosity in digestive tract, possibly because of a more mature microbial population that may help hydrolyze the non-starch polysaccharides (Coon, 2001a). Sreemannarayana et al., 1989 demonstrated a slight increase in relative large intestine weight to body weight of hens from 20 to 58 weeks of age. Bedford (2002) summarized the results from many feed enzyme studies and suggested that if the control diets already had the capacity to cause the bird to approach its genetic potential, then there was little that can be achieved by dietary modification. In experiments with very poor animal performance under control circumstances, enzymes enhance the function of the most limiting nutrients for the animal therefore the improvement in animal's performance can be demonstrated clearly.

Leeson and Summers (2001) mentioned that water intake is closely correlated to feed intake. In the current experiment, the daily water intake of birds fed the corn-soybean diet was significantly higher than that of birds fed the corn starch diet because they consumed more feed, however the water:feed consumption ratio was not significantly different (Table 8). Birds consumed more water and had higher water:feed consumption ratio as the level of defatted rice bran increased in diets. Enzyme supplementation did not affect the daily water intake and the water:feed consumption ratio, while heat stress significantly increased those values.

Feed intake and water intake of birds were affected by heat stress condition. Birds raised in the heat stress condition consumed significantly less feed in order to limit

extra heat production, and consumed more water to fulfill demands for evaporative cooling by panting, consequently, these birds exhibited lower body weight and weight gain. A decline in heat production as a result of lowered feed consumption with increased temperature was demonstrated in studies of Swain and Farrell (1975) and Teeter and Belay (1996). Metabolism of feed declined during heat stress condition due to a reduction of blood flow to the viscera (Bottje and Harrison, 1985; Wiernusz et al., 1992). Because of the decline in feed consumption and an increase in water intake, the water:feed consumption ratio was increased significantly (Table 8, Figure 2).

No differences were detected in the digesta retention time of birds fed different diets (Table 8). An increase in the intestinal retention time of birds fed the high-fat diet that led to the extra metabolic effect was suggested by Mateos and Sell (1980). This may due to the action of cholecystokinin (CCK), a hormone released from the duodenum and the jejunum in response to lipids and amino acids entering into the small intestine (Denbow, 2000). The CCK acts to inhibit gastric emptying and stimulate gall-bladder contraction, pancreatic enzyme secretion, and gastric acid secretion, which subsequently improves digestibility of nutrients and increases metabolizable energy of other feed constituents (Coon, 2001a). Farrell and Martin (1998) found that the relative gut viscosity declined with an increase rice bran inclusion (0, 20, and 40%, respectively). The decrease in gut viscosity may shorten digesta retention time of the high-fat diets. The digesta retention time was significantly longer in birds under the heat stress condition. This was consistent with the observation of Denbow (2000) that increasing in the environmental temperature could slow transit time.

The dietary treatment did not significantly affect percent carcass and carcass composition of birds, except birds fed the corn starch diet that had higher percentage of wings (Table 9 and 10). Heat stress significantly increased percent carcass. The percentage of legs and wings were significantly increased, while the percentage of breast decreased. These results indicate that an increased percentage of carcass was not due to increased protein deposition. Since the feed intake of birds under heat stress condition was reduced, it resulted in a reduction of protein intake (Coon, 2001b). Temim et al. (2000) found that the chronic heat stress markedly reduced the protein synthesis of broilers, leading to reduce protein deposition. The abdominal fat percentage of birds increased significantly with the 25% defatted rice bran inclusion. This may be due to the direct incorporation of fat into the body as a part of growth. Heat stress significantly decreased fat content in the carcass because of lower feed intake. However, this was inconsistent with the study of Swain and Farrell (1975) who reported a significant increase in fat content of the carcasses in birds raised under high temperatures.

Percent intestinal weight, percent of different parts of intestine and morphology of duodenum and jejunum were not significantly affected by dietary or temperature treatments, except for percent duodenum and jejunum. This may be because the non-starch polysaccharide in the defatted rice bran did not cause high viscous condition in the digestive tract, therefore it did not result in a difference in the weight of the small intestine and colon or the morphological change of intestinal lining of the birds. Wang (2000) demonstrated that 50% rice bran inclusion in the broiler chick (5-12 day of age) diet markedly increased the relative size and length of all segments of the digestive tract

with approximately 40% increase in weight and 38% increase in length. Inclusion of an enzyme preparation high in xylanase activity in that diet, significantly decreased the weight of proventriculus (11%), gizzard (13%), colon (8%), and total gastrointestinal tract (7%), while the relative length of digestive tract was unaffected. The viscous environment in the intestinal lumen of chicks causing by water-soluble non-starch polysaccharide also markedly reduced the number of zigzag patterns and ridge-shaped villi, which were commonly found in chicks fed on the control diet, whereas the number of tongue-shaped villi was higher (Langhout et al., 2000). The increase microbial activity in the ileum was increased in broiler chicks fed diet containing high-viscous pectin, while chicks fed diet supplemented low-viscous pectin were not greatly affected (Langhout et al., 1999). In chicks fed high-viscous diet, the number of goblet cells, which are responsible for the secretion of mucin, also increased resulting in decreased nutrient absorption. An increase in maintenance energy requirement for intestinal cell turnover might partially account for the lower growth performance of birds fed diet containing water soluble non-starch polysaccharide. Pell et al. (1992) reported that dietary corn oil and guar gum independently stimulated rat's intestinal crypt cell proliferation. However, the combination of both led to a three- to four-fold increase in colon mucosal. Ritz et al. (1995) found an increase in villi height within the jejunum and ileum of young turkeys fed corn-soybean diet supplemented with amylase. However, no responses were found in poults fed similar diet supplemented with xylanase due to the low level of xylanase supplementation.

In this experiment, percent duodenum of birds was significantly increased while percent jejunum was decreased under heat stress environment. The effects of environment temperature upon intestinal morphology and function have been shown in many species, such as rodents (Carpenter and Musacchia, 1978; Chu et al., 1979), piglets (Dauncey et al., 1983) and broilers (Mitchell and Carlisle, 1992). *In vivo* jejunal uptake of galactose and methionine increased (Mitchell and Carlisle, 1992) as a result of the adaptation to optimize nutrient absorption due to reduced feed intake, decrease in size of the absorptive compartment, and increase in microbial activity. The changes in jejunum mucosal morphology and activity may cause a decrease in percent jejunum weight in this experiment, although no differences were found in villi height, crypt depth, or villi height:crypt dept ratio.

In this experiment, the inclusion of 25% defatted rice bran tended to decrease percent ash and zinc content in the tibia of 3-week old chicks, but these effects were not found in the 7-week-old birds. Percent phosphorus was significantly higher in 3-week-old chicks fed the 25% defatted rice bran diet supplemented with enzyme. Percent calcium of 3-week-old chicks fed the corn-soybean diet and the 25% defatted rice bran diets was significantly higher. Therefore, the calcium:phosphorus ratio in the tibia of 3-week-old chicks was highest in chicks fed the corn-soybean diet and lowest in chicks fed the 10% defatted rice bran diet without enzyme supplementation. In the 7-week-old broilers, percent calcium and the calcium:phosphorus ratio were not affected by dietary treatment. Phosphorus in the tibia was significantly lower in birds fed the corn starch diet and was increased as the level of defatted rice bran increased. Enzyme supplementation

significantly increased percent phosphorus in the tibia only with the 10% rice bran inclusion.

Phosphorus in excreta of 5-week-old broilers increased significantly as level of the defatted rice bran increased, therefore phosphorus retention was significantly decreased as amount of the defatted rice bran increased in diets (Table 18). Enzyme supplementation significantly increased phosphorus retention at the 25% defatted rice bran inclusion by 92.6%, but no difference was found at the low level of inclusion. The effects of phytase to significantly improve the phytate phosphorus hydrolysis and the total phosphorus retention when using the defatted rice bran as the sole source of phosphorus were demonstrated in both broilers and layers (Leske and Coon, 1999)

Percent calcium in excreta significantly decreased when the level of defatted rice bran increased. However, calcium retention did not increase as percent calcium in excreta decreased. This due to the amount of excreta increased in birds fed 25% defatted rice bran diets (Figure 4). When xylanase was added into that diet, the amount of excreta was decreased significantly. This indicated that xylanase improved dry matter digestion of diet. Calcium retention decreased significantly when enzyme was added in the 10% rice bran diet, but was not affected in the 25% defatted rice bran diet. Ballam et al. (1984) studied the effect of fiber source (alfalfa meal, cellulose, and cotton seed hulls) and phytate source (rice bran vs wheat bran) with two levels of calcium and non-phytate phosphorus on phytate hydrolysis in the chicks. Chicks fed diets containing the lower levels of calcium and non-phytate phosphorus hydrolyzed more phytate than those fed higher level, except when the diet contained rice bran. This may imply a greater stability

of rice bran phytate compared to that from other cereal grains. Halpin and Baker (1987) also found that both neutral detergent fiber and ash fractions of rice bran significantly lowered tissue manganese concentrations, but not to the same extent as that caused by rice bran itself. The neutral detergent fiber of other feed ingredients (corn, soybean meal, fish meal, and wheat bran) had very little association with zinc or manganese. These results imply that both phytate and non-starch polysaccharide in rice bran can decrease bioavailability of minerals in diets.

Adding phytase did not only increase the phytate phosphorus utilization, but also released other minerals that complexed with phytate. Zinc can form the most stable complexes with phytate (Cheryan et al., 1983). Therefore at the high level of phytate in the diet, the zinc content in the tibia of 3-week-old chicks tended to decrease. In the current study, including enzyme to the diets increased the tibia zinc content in 3-week old chicks, but the difference was not significant. Adding enzyme in diets containing the defatted rice bran resulted in an increase in tibia manganese content of 3-week-old chicks fed the 25% defatted rice bran diet. However, this effect was not observed in the 7-week-old birds. The investigation of Mohanna and Nys (1999) indicated that microbial and vegetal phytase increased zinc and manganese bioavailability when both minerals were supplied at the minimal level or lower levels of chick's requirement. The positive effect on bone mineral deposition was less apparent when higher levels of zinc and manganese were supplemented in the diets.

The primary objective of using xylanase was to aid in degrading arabinoxylans in defatted rice bran since broilers could not produce endogenous enzyme. By reducing the

amount of undigested non-starch polysaccharide, it could decrease the level of fermentation in small intestine, which are partly responsible for the anti-nutritive activity in chickens (Choct et al., 1996). The effects of using enzyme to hydrolyze non-starch polysaccharide occurred clearly in diets containing highly viscous non-starch polysaccharide such as barley, wheat, or rye. However, non-starch polysaccharide in rice bran is not capable of causing a viscous condition in gastrointestinal tract, therefore the effect of using xylanase to improve rice bran utilization at low levels of enzyme may not be seen in growing birds. Silva et al. (2003) reported the use of three commercial enzymes including an enzyme that contained only phytase activity; an enzyme that contained both xylanase and β-glucanase activities; and a multi-enzyme mixture that contained cellulase, xylanase, β-glucanase, α-amylase, and pectinase activities, in broiler chicks fed diets supplemental rice polishing (20 and 25% in starter and grower diets, respectively). The gut fluid viscosity was not affected by dietary treatment and was considered to be very low (1.20-1.62 mPa). Birds fed on enzyme-containing diets, except for the multi-enzyme mixture, grew faster and had better feed conversion ratio. However, the effect occurred only in the starter period, which was similar to what happened in the current experiment. This may due to the fact that more mature birds can tolerate a high level of non-starch polysaccharide in diets. Petersen et al. (1999) reported an apparent reduction in viscosity with increased age of birds fed barley-based diets. The hindgut digesta viscosity was reduced approximately 50% from birds at 25 to 45 days of age. Since rice bran hemicellulose can bind with some minerals, the use of xylanase should also partly release the minerals from the complexes and improve the bioavailability of minerals.

The feed cost per weight gain of birds was highly associated with feed conversion ratio and price of feed ingredients. The feed cost per pound of defatted rice bran diets was relatively low as levels of defatted rice bran increased because of the low price of defatted rice bran used in this experiment. The feed cost per weight gain of birds fed corn starch diets was highest in every period because the price of corn starch was high. Adding both phytase and xylanase caused a significant increase in overall feed cost per weight gain at 25% defatted rice bran inclusion, but not at 10% rice bran inclusion. Therefore if the price of defatted rice bran is cheap, using defatted rice bran at 25% inclusion without enzyme supplementation should be considered. The heat stress environment caused higher feed cost per weight gain compared to the thermoneutral group and enzyme supplementation was not beneficial.

Conclusion

During the starter period, defatted rice bran diets promoted growth performance of chicks. Supplemental enzyme improved feed conversion ratio of chicks fed 10% defatted rice bran diet. Using high fat to adjust the metabolizable energy of defatted rice bran diets seemed to be beneficial for growth promotion. Therefore, the benefit of added enzyme was not seen. No significant differences were found in carcass yield nor in morphology of the duodenum and the jejunum. Tibia ash and zinc content were not affected by dietary treatment. Tibia calcium, phosphorus, and manganese content of 3-week-old chicks were improved when enzyme was added in 25% defatted rice bran diet. However, only improvement in tibia phosphorus of 7-week-old birds was detected when

enzyme was added in 10% defatted rice bran diet. Phosphorus retention increased almost 100% in birds fed 25% defatted rice bran diet supplemented with enzyme. However, it was still very low. Feed cost per weight gain of birds fed 25% defatted rice bran diets were lower. From the growth performance and feed cost per weight of birds, supplemental enzyme was not beneficial in heat stress environment.

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PART V

SUMMARY AND CONCLUSIONS

The increasing in poultry production following the increase in demands for animal meat, has brought a competition for use of cereal grains as poultry feed ingredients. To decrease the cost of feed production, by-products from agricultural processing manufacturers need to be considered. Rice bran is a major by-product from the rice milling process. It could be used as an ingredient in livestock feed as full-fat or defatted rice bran. Two major limitations for the use of these products are the high amount of phytate phosphorus and non-starch polysaccharides. Poultry do not have endogenous enzymes to digest both substrates. The problems associated with both phytate and non-starch polysaccharides tend to be even worst for poultry production under heat stress conditions. By supplementing exogenous enzymes, the nutritional value of defatted rice bran is possible to be improved.

Therefore, the objectives of this research were to evaluate the production performance of broilers fed defatted rice bran diets treated with commercial enzyme phytase alone or a combination of phytase and xylanase under two different environmental temperatures, thermoneutral and heat stress, and to evaluate the benefit of the enzyme supplementation in order to reduce phosphorus excretion and increase phosphorus availability of broilers. Two experiments were conducted. In the first experiment, six replicate groups of day-old chicks were assigned to five dietary treatments comprised of a control corn-soybean meal diet with 25% corn starch and four diets containing two levels of defatted rice bran (10 and 25%) with or without phytase (500 units/kg diet) supplementation. In experiment 2, five replicate groups of chicks were assigned to dietary treatments similar to first experiment, but using both phytase

(500 units/kg diet) and xylanase (1000 units/kg diet) instead of phytase alone plus an additional basal corn-soybean meal based diet. Available phosphorus in diets containing phytase was calculated to be 0.1% reduction from the requirement for broilers. On day 21, some birds were slaughtered and tibias harvested for mineral analysis. The remaining birds were transferred to either a thermoneutral temperature chamber (23.9^{o}C) or a heat stress chamber (23.9-35^{o}C, diurnal). Digesta retention time was determined and a 5-day total excreta collection was done on 35-day of age. Birds were slaughtered at seven weeks of age, tibias were harvested, and intestines examined.

The use of defatted rice bran up to 25% in broiler diets was not detrimental to broilers in either experiment. The beneficial effect of high fat level may overcome the problems associated with high phytate phosphorus and non-starch polysaccharide in defatted rice bran. Dietary enzyme supplementation did not improve the production performance of broilers, but improved tibia mineral content. This indicates that enzyme supplementation could improve the bone strength of broilers, which is one important factor in broiler production. Broilers have high growth rate, and strong legs are important for weight-bearing. Inadequate mineral bioavailability could cause leg disorders that restrict movement of birds to feeders and drinkers. Not only do these birds gain less weight because of lower feed and water intake, the carcasses of these birds tend to be rejected during processing. Although abdominal fat was increased in birds fed 25% defatted rice bran diets, this type of fat and also fat associated with the skin could be easily removed.

Although phosphorus retention of birds did not improve much by using enzyme, percent phosphorus in excreta was decreased in both experiments in birds fed 25% defatted rice bran diet. This is an important consideration as attempts are made to decrease the environmental problems associated with the high phytate phosphorus content of manure.

Since the price of defatted rice bran was cheaper than other major ingredients, use of 25% defatted rice bran diet lowered feed cost per weight gain of broilers. Use of this product will not only increase the profitability for broiler producers, consumers will also benefit from the lower retail cost of production. As a result, consumers might tend to buy more broiler meat and therefore consume more animal protein.

From the growth performance and feed cost per weight of birds in these experiments, supplemental enzymes did not alleviate the adverse effects of heat stress. The heat stress generated reduction of breast part of carcass, which is of greatest value, was also found in both experiments. However, the use of defatted rice bran diets with high percentage of added fat may help to decrease heat production of birds under heat stress condition. Birds fed defatted rice bran diets also consumed more water, which is important for heat dissipation to maintain normal body temperature, and consequently decreases mortality among birds.

The use of by-products from agricultural processing manufacturers as feed ingredients can lower the cost of production, especially in large scale poultry production. However, the anti-nutritional factors in those by-products have to be decreased to the

levels that would not affect growth performance of the birds. Using exogenous enzymes in poultry diets could improve the nutritional value of many feed ingredients and allow the use of a wide range of ingredients without compromising bird performance, thereby providing great flexibility in least-cost feed formulation. However, types and level of enzyme used in diets as well as cost of feed production have to be considered.

VITA

Ornprapun Puminn was born in Bangkok, Thailand, where she graduated from Kasetsart University and received the Bachelor of Science (Agriculture) with second class honors in 1988. She worked as a secretary to the deputy managing director of Laemthong Farm Co., Ltd. for one year. In 1989, she enrolled in the graduate program of the Animal Science Department, Kasetsart University and received the Master of Science (Agriculture) degree in animal nutrition in 1993. She was employed by Kasetsart University, Kamphaengsaen Campus, Nakhon Pathom in 1993 as an animal husbandry officer and promoted to be an instructor in 1995. Her responsibilities include teaching, conducting research, and extension activities mainly on poultry production and nutrition. In 1999, she received a graduate research assistantship from The University of Tennessee, Department of Animal Science to pursue the Doctor of Philosophy degree in Animal Science. In August of 2003, she completed the requirements for that degree, with a concentration in poultry nutrition. She returned to Thailand in August 2003 where she will resume her career as a faculty member at Kasetsart University, Kamphaengsaen Campus, Nakhon Pathom and serve her home country.